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COMPARATIVE ENZYME CYTOCHEMISTRY OF LYSOSOMAL ACID HYDROLASES IN HOMOLOGOUS TISSUES OF DIFFERENT VERTEBRATE SPECIES - A DESCRIPTIVE AND EXPERIMENTAL STUDY

by

Kurt Herald Weissenborn ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF PATHOLOGY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Comparative Enzyme Cytochemistry of Lysosomal Acid Hydrolases in Homologous Tissues of Different Vertebrate Species - A Descriptive and Experimental Study", submitted by Kurt Herald Weissenborn, in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The histochemical distribution of four lysosomal acid hydrolases, (i.e., acid phosphatase, B-glucuronidase, glucosaminidase, and non-specific esterase), were investigated in six standard tissues of twenty-seven vertebrate species,(i.e., Walley pike, Northern pike, newt, salamander, grass frog, turtle, chameleon, chicken, magpie, robin, horned owl, Japanese quail, rabbit, guinea pig, golden hamster, mouse (Ajax strain), field mouse, rat, cow, sheep, horse, pig, cat, dog, and man).

Lysosomes are most numerous in proximal renal tubules, hepatocytes, phagocytic cells of the reticuloendothelial system, (i.e., Kupffer cells, splenic macrophages, macrophages of lamina propia of small intestine, and perithelial microglia), duodenal absorptive cells and cortical neurons of brain. Lysosomes in different tissues, and in homologous tissues of different species, show considerable variation in size and number. Lysosomal acid phosphatase shows a wide distribution in tissues of different species, but is not invariably present in lysosomes. Inhibitor-resistant esterase, also widespread in lysosomes,is a somewhat less reliable marker enzyme than acid phosphatase. Glucuronidase and glucosaminidase show a variable and restricted occurrence.

Patterns of lysosomal acid hydrolases showed considerable variation between tissues and between species, the latter without apparent relation to taxonomic position. Diffuse cytoplasmic localization of esterase and glucuronidase in certain circumstances, denoted the presence of microsomal isoenzymes. The problem of precision of cytochemical localization and quantitation are discussed in detail.

Modulations of lysosomal acid hydrolase content of renal and hepatic tissues of rat and mouse were induced by the administration of testosterone propionate, oestrone, dextran, glucagon, N-2-fluorenylacetamide, cortisone, phenacetin and phosphorus. A striking increase in microsomal and lysosomal glucuronidase activity in kidney of male mouse was induced by the administration of testosterone propionate, in conformity with a sixteen-fold increase in enzyme activity by biochemical assay.

N-2-Fluorenylacetamide selectively depressed lysosomal acid phosphatase in liver, kidney and absorptive epithelium of rat. Induction of hepatic lysosomes by pinocytosis of dextran, formation of cytolysosomes by glucagon, stabilization of lysosomal membranes by cortisone, labilization of hepatocellular lysosomes in phosphorus-induced necrosis, and phenacetin-induced formation of residual bodies in liver and kidney, failed to produce significant alterations in lysosomal acid hydrolases.

Histochemical methods under the foregoing circumstances offer advantages of precision of localization of enzyme activity, but are relatively insensitive to small quantitative changes in enzyme activity.

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INTRODUCTION

The lysosome, as a new cell organelle, was first described in biochemical terms by de Duve, Pressman, Gianetto, Wattiaux, and Appelmans in 1955. These workers found various hydrolases with acid pH optima in the "light" mitochondrial fraction, concentrated from homogenates of rat liver by differential centrifugation. The five acid hydrolases initially identified were acid phosphatase, B-glucuronidase, cathepsin, acid ribonuclease, and acid deoxyribonuclease, to which have since been added arysulfatase, phosphoprotein phosphatase, B-galactosidase, N-acetyl-B-glucosaminidase, L-mannosidase, and inhibitor-resistant esterase (see Table 1). de Duve and his co-workers also observed that the hydrolytic enzymes of the isolated lysosome fraction were only active biochemically if the granules had been damaged by osmotic shock, freezing and thawing, or by detergents. Latency of enzyme activity was considered to be due to the presence of a limiting membrane barrier of lipoprotein which restricted the accessibility of internal hydrolases to external substrates. Confirmatory evidence for the presence of such a membrane barrier has since been provided by experiments with lecithinase and proteolytic enzymes. After all of these treatments, contained lysosomal hydrolases were found to be released in parallel amounts simultaneously, and in fully active form. The treatments that are now known to affect the structure of lysosomal membrane with release of acid hydrolases are listed in Table 2.

In 1956, lysosomal fractions isolated from rat liver were examined by electron microscopy and were found to contain numerous particles with a single outer limiting membrane, and an electron-dense

Table 1. Enzymes and Other Substances Localized to Lysosomes.

(From Weissmann, 1965).

Enzyme

Acid phosphatase
Acid ribonuclease
Acid deoxyribonuclease
Acid protease (or proteases); cathepsins
Phosphoprotein phosphatase
Beta-glucuronidase
Beta-N-acetylglucosaminidase
Alpha-mannosidase
Beta-galactosidase
Alpha$_{1--4}$glucosidase
Hyaluronidase
Lysozyme
Phospholipase, acid lipase
Phosphatidic acid phosphatase
Collagenase
Aryl sulfatases A and B
Non-specific esterases

Other Substances

Phagocytin
Cationic "inflammatory" protein
Mucopolysaccharides and glycoproteins
Plasminogen activator
Permeability-inducing protease
Hemolysin (or hemolysins)
Unidentified basic proteins

Table 2. Labilzation and Stabilization of Lysosomes.
(From Weissmann, 1965).

In Vitro	In Living Cells
Labilization:	
Hypotonic conditions	Vitamin A
Freezing and thawing	Endotoxin
Low pH	Streptolysins O and S
Nonionic detergents	Ultraviolet irradiation
Digitonin	X-irradiation
Blendorization	2,4-dinitrophenol
Ultraviolet irradiation	Antigen-antibody reactions
X-irradiation	High oxygen excess
Cations (5 Millimolar)	Icterogenin
Phospholipase	Ischemia
Proteases	Anoxia
Staphylcoccal hemolysins	shock
Streptolysins O and S	Prophase of mitosis
Progesterone and testosterone	Endocytosis
DOC	Metamorphosis and tissue resorption
Diethylstilbestrol	
Etiocholanolone and 5 beta-H steroids	Virus infection
5 beta-H bile acids	Starvation
Icterogenin	Thyrotropin in thyroid
Cantharidin	
Vitamin A	
Cysteine	
Lysolecithin	
Stabilization:	
Cortisol	Cortisone and analogues
Cortisone	Chloroquine
Cholesterol	Serum factors in autoimmunity
Chloroquine	"Tolerance" to endotoxins
Antihistamines	
Serum factors in autoimmunity	
Beta-methasone	
Prednisone	

matrix (Novikoff, 1956). Attention was soon directed to the resemblance of these particles to peri-canalicular "dense-bodies" observed by electron microscopists. Confirmation of the identity of peri-canalicular dense bodies with lysosomes was provided by cytochemical localization of acid phosphatase activity in situ in tissues processed for electron microscopy (Novikoff, 1959c).

On the basis of the foregoing correlation, it was possible to define hepatic lysosomes in terms of fine structure, as single-membrane limited dense bodies, 0.5 to 4.0 microns in diameter, composed of electron-dense matrix with enclosed internal vacuoles, ferritin granules, and occasional collections of phospholipid material. Soon after the initial separation of lysosomes from liver by de Duve and his co-workers, granules containing a similar spectrum of acid hydrolases were isolated from homogenates of rat kidney by Straus (1954).

Subsequent cytochemical studies of lysosomes in different tissues by light and electron microscopy revealed considerable structural heterogeneity that appeared to be associated with the functional state of the organelle. The following section deals with the major recognized types of lysosomes and their functions under physiological and pathological conditions.

Primary Lysosomes:

Primary, also called pure, true, and original, lysosomes are those whose enzymes have never been engaged in a digestive event. Their mode of formation is poorly understood. Polymorphonuclear leukocyte granules are considered primary lysosomes in which acid hydrolases are stored until some phagocytic event triggers their release (Hirsch and Cohn, 1964). Novikoff, (1961, 1963, 1964), Novikoff, Essner, and Quintana (1963), and Novikoff and Shin (1964), postulate that acid hydrolases,

produced by biosynthesis in the cysternae of the rough endoplasmic reticulum, are transferred via the smooth endoplasmic reticulum to the Golgi apparatus, concentrated, and released as single-membrane bound vesicles into the cytoplasm to appear as primary lysosomes. Supporting evidence comes from the demonstration of acid phosphatase in the Golgi apparatus and in vacuoles adjacent to Golgi vesicles in which digestive processes were in evidence (Novikoff, 1963; Gordon, Miller, and Bensch, 1965; Novikoff, Essner, and Quintana, 1964; Ericcson, 1964; Miller and Palade, 1964).

More recently, Novikoff (1964), Novikoff, Essner, and Quintana (1964), and Novikoff and Shin (1964), have introduced the additional possibility that lysosomal particles may arise directly from smooth endoplasmic reticulum in the immediate vicinity of the Golgi apparatus as dense bodies and autophagic vacuoles, and the concept is now called the Golgi-Endoplasmic Reticulum-Lysosome System.

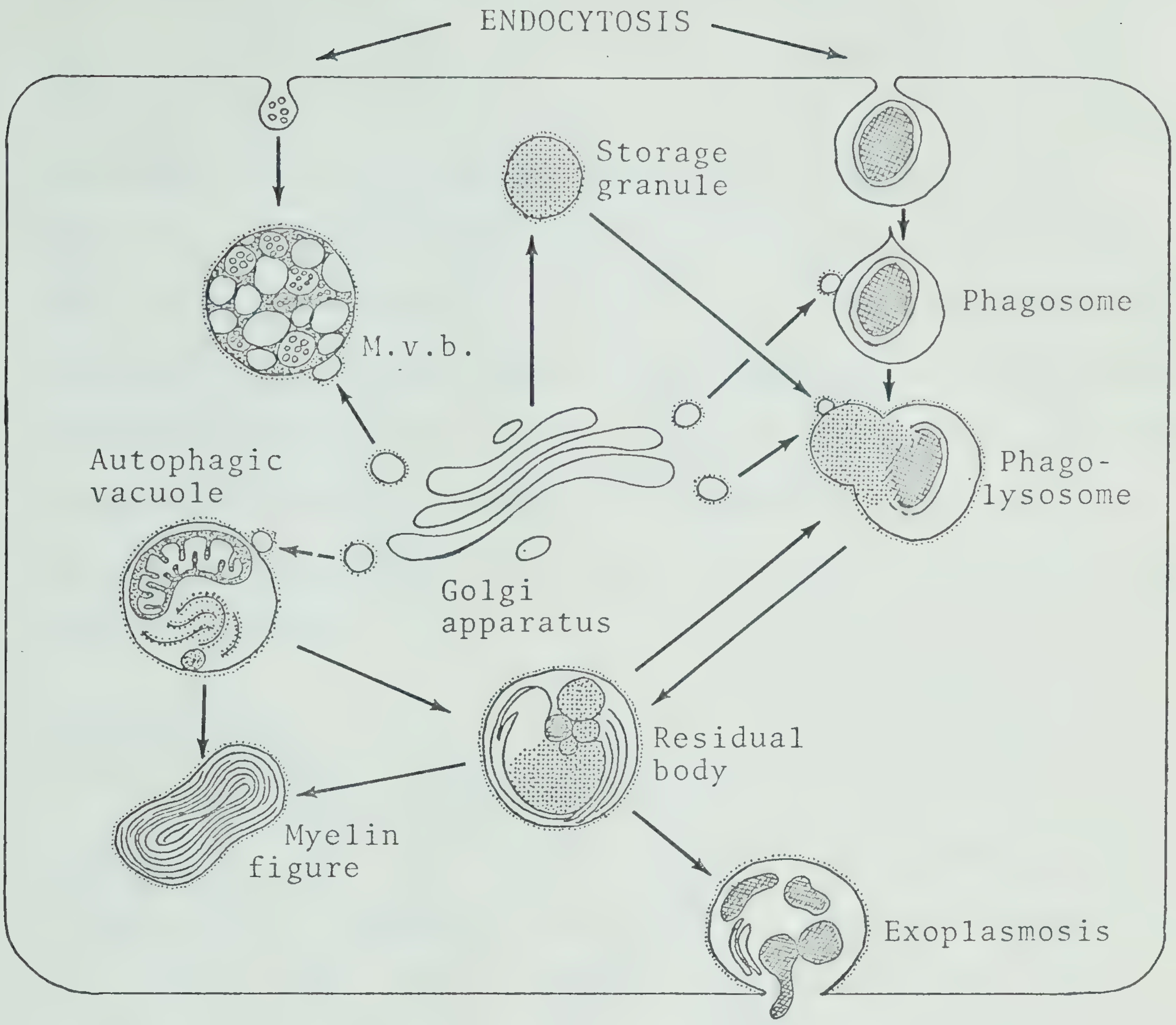
Cytolysosomes:

Large membrane-bound cytoplasmic structures that variously contain mitochondria, endoplasmic reticulum, microbodies, glycogen particles, and cytoplasmic debris, have been observed in diverse tissues under normal and experimental conditions. Novikoff (1960 b) termed these enlarged lysosomes "cytolysosomes". Alternately these have been called cytosegrosomes by Ericcson (1964), and autophagic vacuoles by de Duve (1963). Experimentally, Ashford and Porter (1962) reported the presence of mitochondria within membrane-bound vesicles in liver cells of rats perfused with glucagon. Novikoff and Essner (1962) extended this work by producing similar changes in rat liver cells by the

intravenous administration cf Triton WR-1339 and demonstrating acid phosphatase within these same vacuoles. Novikoff (1963 a) has suggested that cytolysosomes are concerned with the enzymatic degradation of cellular organelles under physiological and pathological conditions. Hruban and Swift (1964) have referred to this process as "focal cytoplasmic degradation".

Phagolysomes; Lysosomes and Endocytosis:

Straus (1954, 1955, 1956) for the first time provided evidence for a relationship between lysosomes and phagocytosed extracellular material when he demonstrated the presence of injected foreign protein (egg-albumin) and two lysosomal acid hydrolases in a purified particulate fraction of rat kidney. Straus (1958, 1959, 1962, 1962, 1963, 1964, 1964), subsequently demonstrated (a) an accumulation of horseradish peroxidase in cytoplasmic granules staining negatively for acid phosphatase, which he termed phagosomes, and (b) at a later stage the presence of both horseradish peroxidase and acid phosphatase in the same vacuoles which he termed phagolysosomes. Inert colloids (Trump, 1961), enzymes (Novikoff, 1960, 1961, 1963), hemcglobin (Erriccson, 1964, 1965; Miller and Palade, 1964; Miller, 1960;), and ferritin (Miller and Palade, 1964), have subsequently been employed to confirm and clarify the work of Straus at both the light and electron microscopic levels. Foreign proteins were shown to be taken up from the lumen of proximal kidney tubules into pinocytic vacuoles pinched off from crypts of the brush border, and transported in these vacuoles toward the basal portion of the cell. It was shown that these pinocytic vacuoles could fuse to form larger vacuoles. In the region of the Golgi complex these pinocytic vacuoles were observed to assume the appearance of phagolysosomes, presumably by fusion with



Text Fig. 1. Nomenclature of Lysosomes and Related Structures. (From Weissmann, 1965).

All structures surrounded by a dotted line have had acid phosphatase demonstrated within them by electron microscopy.

endocytic, acid hydrolase-containing primary lysosomes.

Carbon and dextran (Meijer and Willighagen, 1961; Daems 1961, 1962; Daems and Persijn, 1964), polyvinylprolidone (Meijer and Willighagen, 1961, 1963; Hübner, 1962), sucrose and other sugars (Maunsback, Madden, and Latta, 1962; Trump and Janigan, 1962; Brewer, 1963, 1964), and iron (Kent et al, 1963), have all been used to further demonstrate the eventual presence of these substances in phagolysosomes in kidney as well as other tissues. Exogenous materials that have been incorporated into phagolysosomes are destined either for digestion or storage, and neutilization. Phagolysosomes may participate in more than one digestive event.

Residual Bodies:

Once digestive processes have been carried to completion in phagolysosomes, these often contain residual membrane fragments, myelin figures, ferritin granules, and other debris. Burned out phagolysosomes are termed "residual bodies" (Novikoff, 1963; de Duve, 1963). These often display morphological and tinctorial characteristics of lipofuscin pigment.

Physiological Necrobiosis and Autophagy

There is growing evidence that processes of physiological necrobiosis and autophagy are in part, at least, mediated by lysosomes. The following are presented as examples:

Regression of the Mullerian Duct:

Brachet, Decroly-Briers, and Hoyez (1958) showed the total and free activities of acid phosphatase, cathepsin, and ribonuclease, increased in male chick embryos during the regression of the Müllerian duct between the ninth and thirteenth day of development. Scheib (1964) observed an

increase in the number and size of lysosomes in the regressing epithelial cells and a concurrent increase in the number of macrophages in the connective tissue.

Regression of the Tail of Xenopus Larvae:

Weber (1963) reported that the amount of cathepsin markedly increases during regression of the tail, a process which is under the control of the thyroid hormone. Cytochemically, Salzmann and Weber (1963) demonstrated most of the lysosomes in the regressing tail to be in macrophages. Eckhout (1964) reported an increase of several lysosomal enzymes during the regression of Xenopus tails. Lysosomes of the macrophages probably function in the clearing of cellular debris.

Post-Partum Involution of Uterus and Regressive Changes in the Ovaries and Endometrium During the Oestrus Cycle:

Acid phosphatase was used as the marker lysosomal enzyme in a cytochemical study of post-partum uterine involution in rats by Lobel and Deane (1962). While little activity was found in virgin and pregnant rat uterus, a marked increase in acid phosphatase activity was noted immediately post-partum in sites of placental detachment, epithelial cells, macrophages, and stromal cells of uterus. The lysosomal function was postulated to be in the resorption and reorganization of the tissues during post-partum involution. Lysosomal changes in ovaries and endometrium during the oestrus cycle were investigated by Barron, Brandes, and Frost (1964). Involuting corpora lutea were found to be surrounded by macrophages containing large numbers of lysosomes and little lysosomal activity was found in lutein cells themselves. Lysosomes of regressing cells during follicular atresia and of the endometrium during the oestrus cycle showed an increase in size and activity related to the lytic process.

Holocrine Secretion in Sebaceous Glands:

Holocrine secretion in sebaceous glands was investigated histochemically at the electron microscopic level by Brandes, Bertini and Smith (1965) who found that with maturation of the cells, the numbers of lysosomes increased greatly, seemingly coming from small vesicles of the Golgi apparatus. Lysosomes released their contents into the general cytoplasm during the disintegration of the mature cells.

Lysosomes and the Thyroid Gland:

Wollman et al (1964), demonstrated a possible function of phagolysosomes in thyroid cells in the degradation of the endogenous protein thyroglobulin. These authors showed that colloid material containing thyroglobulin is ingested from the lumen of the thyroid follicle into thyroid cells by pinocytosis. Accumulation of ingested material occurs in the apical cytoplasm in droplets or phagosomes. Formation of these phagosomes was induced by Wollman et al (1964), by the injection of thyroid stimulating hormone (TSH) into hypophysectomized animals. T.S.H.-induced phagosomes were deficient in acid phosphatase activity. At a subsequent stage acid phosphatase was found to be present in these colloid-containing vacuoles indicating that phagolysosomes had been formed. The lysosomal acid hydrolases were postulated to function in the splitting of thyroxine hormone from thyroglobulin. Wetzel, Spicer, and Wollman (1965) confirmed the foregoing sequence of events at an ultrastructural level.

Lysosomes and Iron Storage:

Ferritin is a frequent constituent of lysosomes, particularly in the liver, and in cells of the reticulo-endothelial system. Daems (1962) suggested that iron that enters liver cells is converted within lysosomes

to a form that is capable of combining with apoferritin, to form ferritin. Hemosiderin granules are composed of large aggregates of ferritin molecules and thus constitute a more concentrated form of iron storage (Richter, 1957, 1958). Excess accumulations of iron may occur under pathological conditions such as hemochromatosis and hemosiderosis. Lysosomes constitute the principal storage site for iron in these conditions (Essner and Novikoff, 1960 a).

Bone Resorption:

Scott and Pease (1956), Gonzales and Karnovsky (1961), and Hancox and Boothroid (1961), found by electron microscopy that osteoclasts contained numerous phagocytic vacuoles and appeared to be active in phagocytosis. Acid phosphatase activity was found to be marked in osteoclasts and chrondrioclasts (Schajowicz and Cabrini, 1958), and later work by Hankelman, Morse and Irving (1964) localized high acid phosphatase activity in a junctional band between osteoclasts and bone. Lysosomal enzymes were found to be released into the external medium during resorption of bone cells in tissue culture under the influence of parathyroid hormone (Vaes, 1965). From the foregoing it is believed that osteoclast lysosomes are active in bone resorption either by the release of lysosomal acid hydrolases from the cell surface, or as phagolysosomes, or both.

Lysosomes and Mitosis:

Observations of mitosis in cultured cells (Robbins and Gonatas, 1964), in hepatic regeneration in rat (Allison and Malluci, 1964; Kent et al, 1965) and in mammalian lymphocytes in culture (Hirschhorn et al, 1964, 1965; Hurvitz and Hirschhorn, 1965), have all described the migration of lysosomes from their usual circumferential position in the cytoplasm to the perinuclear region during prophase. Cultured lymphocytes,

normally almost devoid of lysosomes, in the presence of phytohemagglutinin undergo transformation to large granule-filled cells before mitosis, these granules migrating from the peripheral cytoplasmic region to the perinuclear region prior to mitosis. Histochemically and biochemically these new structures were identified to be lysosomes (Allison and Malluci, 1964; Hirschhorn et al, 1965). Other non-specific stimulants for lymphocyte mitosis in culture, e.g. Streptolysin S, as well as specific antigens to which the lymphocyte donors had previously been sensitized, also induced the formation of lysosomes. The close apposition of lysosomes to the nuclear membrane during prophase suggest that lysosomes may participate in cellular remodelling prior to cell division.

Lysosomes and Immunity:

Antigenic macromolecules enter reticulo-endothelial cells in phagosomes and subsequently appear in phagolysosomes in which catabolism takes place (de Duve and Wattiaux, 1966). Antigenic Streptococcal M protein (Gill and Cole, 1955), P^{32} labelled bacteria (Cohn, Hirsch and Wiener, 1963), I^{131} labelled albumin (Mego and McQueen, 1965), and antigenic bacteriophages (Uhr and Weissmann, 1965), have been shown to be sequestered and degraded in lysosomes of phagocytic cells. Uhr and Weissmann (1965) have shown materials from lysosome-rich fractions of such cells to be immunogenic.

Fishman and Adler (1963) have theorized that the immunogenic complexes of antigen fragments and ribonucleic acid produced in phagolysosomes are transferred to antibody-producing cells. Transfer of antigenic determinants from macrophages to antibody-producing plasma cell precursors is believed to occur via cytoplasmic bridges shown to exist by Schoenberg, Mumaw, Moore and Weissberger (1964), between phagocytic

and antibody-producing cells in lymph nodes and spleen -- especially in immunized animals. Similar associations between plasma cells and macrophages in the lamina propria of the gut have been demonstrated by Deane (1964).

Pinocytotic uptake of antigen-antibody complexes occurs readily in eosinophils (Sabesin, 1963), spleen cells (Patterson et al, 1962), and tissue culture cells (Tahaba, Kinuwaki, and Hayashi, 1964). The formation of phagolysosomes and the degradation of these complexes in phagocytic cells probably obviates further cellular injury that they may cause (Straus, 1967).

Pathological Changes Related to Lysosomes and Phagosomes

Cell Injury and Death:

Participation of lysosomes in pathologic cell injury and cell death represents an exaggeration of one of the known physiological functions of this organelle. Induced cellular injury has been reported by Hruban et al (1963), to produce focal areas of cytoplasmic degradation in kidney, liver and pancreatic cells after potassium deficiency, partial occlusion of renal vein, and injection of metabolic antagonists. Ligation of the ureter produced enlargement of lysosomes in kidney cells (Novikoff, 1959). Barron and Sklar (1961) report enlargement of lysosomes in nerve cells after axon sectioning. Whether the foregoing modulations in lysosomes represent primary or secondary alterations is still not certain.

Exaggerated Formation of Phagolysosomes Under Pathologic Conditions:

Trowell (1946) produced "watery vacuoles" in liver cells by increasing sinusoidal pressure and anoxia. These vacuoles are likely synonymous with the phagolysosomes. Doniach and Weinbren (1952), Aterman (1958), Nairn, Chadwick, and McEntegard (1958), Anderson, Cohen, and

Barka (1961), and Schlicht (1963), demonstrated the presence of injected substances, as well as serum proteins, in such liver cell vacuoles. More recently Daems (1962) and Kent, et al., (1963 a, b), have studied the formation of enlarged liver cell vacuoles by injecting dextran and iron-dextran complexes. Similar studies employing mercury sulfide (Oudea, 1963) and sucrose (Wattiaux, et al., 1964; and Brewer and Heath, 1963), have been conducted on these vacuoles. In kidney cells vacuole formation of a similar type was observed after the injection of sucrose (Janigan and Santamaria, 1961, Trump and Janigan, 1962; Maunsbach, Madden, and Latta, 1962; and Straus, 1961, 1963, 1964 a, b).

Storage Diseases Produced by Deficiencies of Specific Lysosomal Hydrolases

Glycogen Storage Disease Type II: (Pompe's Disease)

Hers, et al., (1963) and Baudhuin, et al., (1964) were the first to identify glycogen storage disease Type II as an inborn lysosomal disease in which L-glucosidase, capable of degrading glycogen, is entirely absent from tissue.

Metachromatic Leukodystrophy

Metachromatic leukodystrophy is a storage disease resulting from a deficiency of Arylsulfatase A, a lysosomal enzyme (Austin, et al., 1964; and Mehl and Jatzkewitz, 1963). Accordingly, cerebroside sulfates accumulate in tissues, particularly of the central nervous system. A deficiency of sulfatase in the urine is of assistance in the clinical diagnosis of cases.

Hurler's Syndrome (Gargoylism)

Heparin sulfate and chondroitin sulfate B accumulate in various tissues and abnormal lysosome-like vacuoles become filled with

stored material (van Hoof and Hers, 1964). At present no specific lysosomal enzyme deficiency has been detected (Hers, 1965), although the foregoing seems plausible.

Biochemical Properties of Lysosomal Enzymes

Biochemical evidence, scattered throughout the literature, indicates that lysosomal hydrolases are widely distributed in tissues throughout the principal phyla of the animal kingdom (Shibko, et al., 1963; Beaufay, et al., 1957; de Duve 1953, 1955, 1959; Stein and Fruton, 1960; Holter, 1954; Roy, 1955; Conchie, 1959; Cohn and Hirsch, 1960). The properties of these enzymes have been reviewed by Woessner (1965). Sumner and Myrbäch (1951), Burstone (1962), Barka and Anderson (1963), and Gahan (1967).

Acid Phosphatase (E.C. 3.1.3.2)

This enzyme catalyzes the hydrolysis of orthophosphoric monoesters to alcohol and phosphoric acid at acid pH according to the following formula:- ORTHOPHOSPHORIC MONOESTER + $H_2O \rightarrow$ Alcohol + H_3PO4.

Acid phosphatase exists in three forms:-

(a) Phosphomonoesterase Type II - is widely distributed in nature. In mammals it is most abundant in spleen, liver and especially abundant in prostate gland. This isozyme has a pH optimum in the range of 5.0 to 6.0 Fluorides and oxalates are strongly inhibitory, while magnesium ions have no effect on its activity.

(b) Phosphomonoesterase Type III is more restricted in its distribution and in mammals occurs only in trace amounts, in liver and spleen. The enzyme has a pH optimum of 3.4 to 4.2, is inhibited by magnesium ions and is rapidly inactivated in neutral solutions.

(c) Phosphomonoesterase Type IV occurs in erythrocytes and seminal

plasma of animals. It hydrolyzes L-glycerophosphate more readily than B-glycerophosphate at a pH optimum of 5.2 to 6.2. The enzyme is readily inactivated by formalin fixation.

B-glucuronidase (E.C. 3.2.1.31)

The hydrolysis of conjugated glucuronides is catalyzed by B-glucuronidase (B-D-glucuronide glucurono hydrolase) as shown in the following general formula:-

B-D-glucuronide + $H_2O \rightarrow$ Alcohol + D-glucuronic acid.

The enzyme also acts as a transferase when, in the presence of a suitable acceptor alcohol, it facilitates the transfer of the glucuronyl group to form an alcohol glucosiduronic acid. This function is believed to be more important than its hydrolytic activity (Fishman, 1961). B-glucuronidases are widely distributed in animal tissues and in bacteria. The function of B-glucuronidase is not as yet clear. However, it is reported to be active in drug and hormone metabolism, a subject that has been recently reviewed in detail by Fishman (1961). The optimal pH for the enzyme is approximately 5.0 and it is specifically inhibited by saccharo - 1, 4-lactone.

N-acetyl-B-glucosaminidase (E.C. 3.2.1. - or 3.2.1.29)

B-glycosides of N-acetyl - D - glucosaminopyranosides are hydrolyzed by the enzyme B-glucosaminidase. Sellinger, et al., (1960) has associated B-glucosaminidase enzyme activity with lysosomes. Distribution of B-glucosaminidase in mammalian tissues and species is wide. Testis, liver, lung, heart, brain, muscle, and other tissues show enzyme activity. Epididymis contains particularly high levels of activity (Findlay and Levvy, 1960). The pH optimum of the enzyme is about 4.5. N-acetylglucosaminolactone has been reported to be a specific inhibitor of B-glucosaminidase (Findlay, et al., 1958). The function of the enzyme

is unknown, although, it is speculated to play a part in "mucoid" metabolism and hydrolysis of degradation products of hyaluronic acid.

Arysulfatases:

Arylsulfatases (arylsulfate sulphohydrolases, E.C. 3.1.6.1) catalyze the hydrolysis of arylsulfates and indoxyl sulfates (Boyland, et al., 1956) according to the following general formula:-

$R\text{-}OSO_3\ H + H_2O - ROH + 2H^+ + SO_4$

Arysulfatases are widely distributed in plants and animals, but their physiological role is not known. Roy (1960) regards arylsulfatases as lysosomal enzymes and suggests their importance to be not in the metabolism of the sulfate moiety, but rather to be in the metabolism of the phenolic moiety of the substrate molecule. The pH optimum of arysulfatases varies with the substrates employed, the tissue, and the buffer. Huggins and Smith (1947), using body fluids and homogenates of tissue obtained a pH optimum of 6.12. Roy (1953) determined the pH optima of ox liver sulfatase to be 5.0 and 6.0 in the presence of acetate buffer. Maengwyn-Davies and Friedenwald (1954) using rabbit liver and kidney, report a pH optimum of 6.0 to 6.1. Arylsulfatases of mammalian livers consist of isozymes types A, B, and C, which are differentiated on the basis of rate of hydrolysis of dipotassium - 2 - hydroxy - 5 - nitro phenyl sulfate (nitrophenylsulfate), and p-nitrophenylsulfate. Isozymes A and B hydrolyze nitrophenylsulfate rapidly and p-nitrophenylsulfate slowly while type C isozyme of arylsulfatase hydrolyzes p-nitrophenylsulfate rapidly. Phenylhydrazine, sodium cyanide, silver nitrate, and semicarbazide strongly inhibit these enzymes.

Aliesterases:

Aliesterases encompass both non-specific esterases and lipases.

Non-specific esterases hydrolyze simple, short-chain, aliphatic esters with fewer than twelve carbon atoms. The enzymes are widely distributed in tissues and show great variation in their pH optima (range 5.0 to 9.0). Their physiological function at present is uncertain. Organophosphorus sensitivity has been used to subdivide non-specific esterases into types A and B. The E 600 (diethyl - p - nitrophenylphosphate) resistance of type A enzyme to 10^{-3} M E600 distinguishes the enzyme from type B, which is sensitive to 10^{-8} M of E600. Organophosphorus-resistant non-specific esterases are associated with lysosomes (Shnitka and Seligman, 1961). As many as thirteen isozymes of non-specific esterases have been demonstrated by starch and acrylamide gel electrophoresis. Lipases differ from non-specific esterases in that they hydrolyze fatty-acid esters with long carbon chains, although their separation on this basis is not complete.

Other Lysosomal Enzymes

Deoxyribonuclease (Deoxyribonucleate oligo-nucleotide hydrolase, E.C. 3.1.4.5)

Two isozymes of this enzyme, DNAase I and DNAase II, or acid DNAase, in the presence of Mg^{++}, Mn^{++}, Co^{++} and Ca^{++} ions catalyze the hydrolysis of DNA, yielding 5'-monophosphate esters and 3'-monophosphate esters respectively. Acid DNAase is believed to be lysosomal in origin (de Duve, 1959), and has a pH optimum (Crystalline DNAase from pancreas) of 7.0.

Acid Ribonuclease (E.C. 2.7.7.16 - Bovine pancreas)

RNA 3', 5' - phosphodiester linkages are attacked by RNAase first by non-hydrolytic alteration of structure and then by enzymatic hydrolysis to the final 3' phosphate nucleoside residue. The enzyme is believed to be lysosomal (de Duve, 1959), and in animals is specific for

pyrmidine nucleoside linkages, displaying a pH range of activity from 5.0 to 9.0 and an optimal pH of 7.0.

B-galactosidase:-

B-galactosidase, which hydrolyzes B-D galactosides (also hydrolyzed by B-glucosidase), α - L arabinosides, lactose, and heptosides, was associated with lysosomes by Sellinger, et al., (1960). High enzyme activity is reported in tissues of various species of animals by Wallenfels and Malhotra, (1960) and Maio and Rickenberg (1960) who also report a low optimum pH for the enzyme.

Phosphoprotein phosphatase:-

This enzyme liberates, by hydrolysis at the optimum pH of 5.5, inorganic phosphate from casein, and other phosphoproteins such as ovalbumin and phosvitin (Norberg, 1950 b; Perlmann, 1952; Schmidt and Laskowski, 1961). Generally wide in tissue distribution, the enzyme shows high activity in brain, adrenal gland, and kidney, and low activity in erythrocytes, skeletal and cardiac muscle.

Cathepsins:-

Cathepsins are proteolytic enzymes that hydrolyze protein and amino acid compounds. They are subdivided into exopeptidases, i.e., aminopeptidases and carboxypeptidases, which hydrolyze peptide bonds adjacent to terminal L-amino or L-carboxyl groups, and endopeptidases which hydrolyze centrally located peptide bonds, as well as terminal peptide bonds (Bergmann, 1942). Endopeptidases include pepsin, trypsin, chymotrypsin and cathepsins.

Cathepsins have been localized to lysosomes by de Duve, et al., (1955). These enzymes are subdivided into cathepsin I, II, III, IV and V. Cathepsins I, II, and III are endopeptidases, with a pH optimum of 5.6. Cathepsin I has a substrate specificity similar to that of pepsin, and

cathepsin II has a substrate specificity similar to that of trypsin. Cathepsin III has an amino peptidase action and may be homeospecific with the metal-activated isozyme of leucine aminopeptidase. Cathepsin IV has a carboxypeptidase activity similar to that of pancreatic carboxypeptidase. Cathepsin V acts on some of the substrates for crystalline chymotrypsin.

Cathepsins are widely distributed in animal tissues and display a great variation between organs. High activity is located in spleen, liver, kidney, and low activity is found in skin, lung, heart, muscle, uterus, and brain. Available histochemical methods for these enzymes lack precision of localization and sensitivity.

Enzymic Histochemical Methods for Demonstrating Lysosomes

A. Acid Phosphatase

There are presently three methods available for the demonstration of acid phosphatase: (a) the lead sulfide method of Gomori (1952), (b) the post-coupling azo-dye method of Rutenburg and Seligman (1955), and (c) the simultaneous-coupling azo-dye method with either phosphate esters or Naphthol AS derivatives (Burstone, 1958), or α-naphthylphosphate (Seligman and Manheimer, 1948; Grogg and Pearse, 1952; Burton, 1954; Barka, 1960 b; Barka and Andersen, 1962). The widely applied Gomori reaction utilizes the substrate sodium glycerophosphate in the presence of lead nitrate at pH 5.0 in 0.05 M acetate buffer. The phosphate, split from the substrate by acid phosphatase, is precipitated as insoluble lead phosphate, and is for optical microscopy made visible by conversion of the white lead phosphate to black lead sulfide, by exposure to a dilute solution of ammonium sulfide. Electron microscopy, dependent on electron opacity and crystalline structure, utilized the initial lead phosphate for localization of acid phosphatase

activity in cells. The Gomori reaction is subject to some disadvantages, such as the affinity of some tissue and cellular components for lead giving rise to non-enzymatic staining, and non-specific nuclear staining believed to be non-enzymic in nature (Bitensky, 1962, 1963 a; Gomori, 1954; Holt, 1959).

The post-coupling method of Rutenburg and Seligman (1955) is theoretically more suitable for histochemical localization of acid phosphatase activity since it allows the reaction to procede at the pH optimal for the reaction at each stage. Diazonium salt-inhibition of the enzyme (Burstone, 1962; Lodja, et al., 1964), is not a problem. Practically, however, the method is subject to diffusion of the primary reaction product, specific affinity of some tissue components for the 6-benzyl-2-naphthol moiety of the substrate, and the lipophilic nature of the end-product, all factors active in nullifying the advantages of the technique.

Simultaneous-coupling azo-dye techniques are presently the methods of choice for acid phosphatase localization. Burstone (1958, 1961) utilized derivatives of hydroxynaphthoic acid anilides for the demonstration of acid phosphatase histochemically. Coupling of the substrates occurred efficiently at acid pH and good localization of activity was achieved.

The method of Seligman and Manheimer (1958) employing α - Naphthyl phosphate as the substrate is essentially like the method of Burstone (1958, 1961). Most diazonium salts will couple with α - Naphthol at a suitable and sufficiently rapid rate at an alkaline pH. Grogg and Pearse (1952) found Fast Garnet GBC to be the most efficient coupling agent at pH 5.0, while Davis and Ornstein (1959) considered diazotized pararosanilin to give better results as a coupling agent. The rate of hydrolysis of the substrate at pH 5.0 to 5.5 is faster than the rate of the coupling reaction, consequently a pH of 6.0 to 6.5 is chosen to get adequate coupling and to capitalize on

the reduced diffusion of the α - Naphthol at this pH. Impurities in the diazonium salts, presence of any incompletely diazotized pararosanilin, the unphysiological pH, and the inhibitory effect of the diazonium salt on the enzyme, all appear to be disadvantages of the technique, limiting its utilization to tissues with generally high acid phosphatase activity.

Barka and Anderson (1963) modified Burstone's (1958) method by using hexazonium pararosanilin as the coupling agent replacing the diazonium salt used. High substantivity, very low solubility of the Naphthol AS derivatives released, and the adequate reaction at pH 5.2, with no diffusion of the end product, make this technique very suitable for acid phosphatase demonstration in situ.

B. B-Glucuronidase

Earlier histochemical methods for B-glucuronidase used azo-dyes (Friedenwald and Becker, 1948; Seligman, et al., 1949; Pearse, 1960), or a Prussian Blue reaction (Friedenwald and Becker, 1948; Fishman and Baker, 1956). More recently Fishman, et al., (1964 a, b), Hayashi (1964) and Hayashi, et al., (1964), utilized the substrate Naphthol AS-BI-B-D-glucosiduronic acid for the demonstration of B-glucuronidase. Naphthol AS-BI-, yielded on hydrolysis of the substrate by the enzyme, is coupled to hexazonium pararosanilin, to form a red pigment residue at the site of enzyme activity. Although little is known about this group of enzymes, Hayashi, et al., (1964), reports different chromatographic properties for some of the isozymes, and both microsomal and lysosomal B-glucuronidases with different pH optima are reported by Davis and Ornstein (1959).

C. N-acetyl-B-Glucosaminidase

A simultaneous-coupling method using the substrate α -Naphthyl-

N-acetyl-B-D-glucosaminide and the diazonium salt Fast Garnet GBC (diazotized O-aminoazo-toluene) was first reported by Pugh and Walker (1959, 1961) for the enzyme B-glucosaminidase. Only moderately accurate localization was achieved with this method. It was further modified and improved by Pugh and Walker (1961) by using the substrate Naphthol-AS-LC-N-acetyl-B-glucosaminide. Subsequently, Hayashi (1964) employed the substrate Naphthol-AS-BI-N-acetyl-B-glucosaminide for the histochemical demonstration of B-glucosaminidase by a simultaneous-coupling reaction using hexazonium pararosanilin or Fast Garnet GBC at pH 5.2 as the coupling reagent. Hayashi obtained precise, granular staining of enzyme activity with both diazonium salts, hexazonium pararosanilin providing less background staining.

Fishman and Goldman (1965), considering the limitations of Hayashi's method, the inhibition of B-glucuronidase by the hexazonium pararosanilin (Watanabe and Fishman, 1964), the slow hydrolysis of the substrate molecule, and the unfavorable pH of the reaction, proposed a post-incubation-coupling technique for unfixed tissue for the demonstration of B-glucuronidase employing Naphthol-AS-BI-B-D-glucosiduronic acid as the substrate and one of a number of diazonium salts as coupling agents (Fast Garnet GBC, Fast Dark Blue R, Fast Blue RR, Fast Blue BB, Fast Red Violet BB, or Fast Bordeaux OL). With this method the pH of the incubation reaction medium could be optimal for the enzyme and the pH of the coupling reaction medium could be optimal for the coupling reaction. The avoidance of the azo-dye enzyme inhibition and the use of a readily hydrolyzed substrate increased the staining obtained in unfixed sections. Localization is not as precise as in Hayashi's method.

D. Aryl sulfatases

Aryl sulfatases exist in mammalian liver and kidney as types

A, B, and C. Types A and B are believed to be lysosomal and possess pH optima of 6.0, type C is microsomal and had a pH optimum of 8.0. On the basis of pH optima Ruterburg, et al., (1952), demonstrated a reaction for sulfatases in lysosomes of kidney using 6-benzoyl-2-naphthyl sulfate as the substrate and a diazonium salt in a simultaneous-coupling reaction.

Woohsman and Hartrodt (1965), based on the insolubility of Naphthol AS compounds and their superior localization, demonstrated arylsulfatase activity in uterus and kidney or rat using the substrates Naphthol AS-BI-sulfate and Naphthol AS-OL-sulfate. Goldfischer (1965) developed a Gomori-type reaction for arylsulfatase employing p-nitrocatechol sulfate as the substrate in the presence of lead nitrate. The enzymically released sulfate of the substrate is captured by lead ions to yield insoluble lead sulfate, which, upon exposure to yellow ammonium sulfide, is converted to lead sulfide. Goldfischer obtained two optimal pH levels of activity, one at pH 4.2 believed to be sulfatase A in glia, pericytes, stellate neurones, granular cells of cerebellum, sheath cells of spinal ganglia, renal glomeruli, endothelial cells, collecting ducts, bile ducts and endothelium of hepatic arteries and veins. These tissues also stained at pH 5.5. Tissues only staining at pH 5.5, believed to be sulfatase B, were Purkinje cells of rat cerebellum, neurones of dorsal root ganglia, and hepatic and renal parenchymal cells. Thomson and O'Connor (1963) believe this reaction to be unreliable in rat kidney and uterus.

E. Esterases

The methods for the histochemical demonstration of non-specific esterases can be grouped on the basis of substrates employed. Thus there are (a) the Naphthol acetate and substituted Naphthol-AS-acetate substrate

employing methods, (b) the thiolacetic acid substrate-employing method and (c) the indoxyl acetate and substituted indoxyl acetate substrate methods. The indoxyl acetate method of Holt (1958), employing the indoxyl acetate or substituted indoxyl derivatives, relies on the esterase to split the substrate, freeing the soluble indoxyl, which is oxidized via its leuco-derivative to the corresponding indigo dye. 5-Bromoindoxyl acetate and 5-bromo-4-chloroindoxyl acetate are two of the more precise substrates. The formation of indigo dye is apparently dependent upon the denaturation and exposure of functional groups of proteins to which indoxyl groups can bind and become oxidized. The denaturation of protein is accomplished by fixation of tissue with formalin. The presence of unfixed protein will not facilitate the binding of indoxyl groups, consequently these are not oxidized readily and indigo dye products are formed sparsely. Localization of non-specific esterase activity to lysosomes in formalin fixed tissue is believed to be due to the selective inhibition of cytoplasmic esterase by formalin fixation and the presence of the ferri-ferrocyanide redox system employed in the method (Shnitka and Seligman, 1961). The Holt (1958) method has been modified by Delellis and Fishman (1965) to a simultaneous-coupling method using the substrate 5-bromoindoxyl acetate and hexazotized pararosanilin as the coupling reagent.

The liberation of hydrogen sulfide from the esterase hydrolysis of thiolacetic acid is the basis for the thiolacetic acid method. In the presence of lead mitrate, hydrogen sulfide, where it is enzymatically released, will react with lead nitrate to form lead sulfide.

The presence of organophosphorus-resistant esterase in lysosomes of kidney, as demonstrated by Pearse (1960), Burstone (1962), and Wachstein,

et al.,(1961), and the presence of diisopropylphosphofluoride-resistant esterase also in kidney lysosomes as shown by Ostrowski, et al.,(1964), has lead Hess and Pearse (1958) to suggest this activity to be due to cathepsin C. Holt (1958) considers this activity to be due to cathepsin D.

The Biochemical Distribution of Lysosomal Acid Hydrolases.

Reports on the biochemical distribution of lysosomal acid hydrolases in various animal species are sparse. Most available reports in the literature deal with the activities of lysosomal acid hydrolases in the liver and kidney of the rat or mouse. Other species of animals, and other organ systems, have received very little attention to date.

It is difficult to compare reported specific activities of lysosomal enzymes of various organs in different species, because diverse methods have been employed in obtaining recorded values. Moreover, some authors report total enzyme activity as μ moles /mg. N. /min. x 10^3, while others report specific activities of enzyme in m μ moles of substrate hydrolysed / min. / mg. of protein, while still others report enzyme activity in terms of μ moles / hour / g. of wet tissue or μ moles / hour/ mg. N. A further difficulty in comparing reported data arises from the use by various groups of workers of different substrates to determine enzyme activity.

Biochemical data on lysosomal acid hydrolases, detailed in Appendix I, suggests heterogeneity of lysosomes, in terms of their acid hydrolase content, and probable function in different tissues. This view is supported by Rahman, et al., (1967) who separated lysosomal fractions from homogenates of rat liver by zonal gradient centrifugation, and carefully investigated the biochemical distribution of marker enzymes for lysosomes (i.e., acid phosphatase, acid ribonuclease, cathepsin C, and cathepsin D), for microsomes (i.e., glucose-6-phosphatase), for microbodies (i.e., urate oxidase),

and for mitochondria (i.e., cytochrome oxidase or succinate dehydrogenase). The results obtained suggest that two different classes of lysosomes exist in rat liver :- one class possesses acid phosphatase and cathepsin C activities, the other possesses acid ribonuclease and cathepsin D activities. Due to shortcomings of the biochemical techniques, however, the authors state that the questions of whether differences in enzyme distribution represent true differences in particulate composition within a given cell type, or whether they represent intercellular differences among various parenchymal cells of the liver lobule, cannot be resolved. An alternate idea that two types of lysosomes may be derived respectively, from cells of liver and from Kupffer cells, in parallel with a suggestion by Browser and de Duve (1967) for rat spleen, is tenable.

The Histochemical Distribution of Lysosomal Acid Hydrolases.

Lysosomes initially were identified in terms of their biochemical properties in the L-fraction of liver cell homogenates (de Duve et al., 1955, and de Duve, 1959). These particles contained a number of acid hydrolases that could be activated by damage to the lysosomal membrane. Histochemical identification of lysosomes in various tissues has been largely based on the use of acid phosphatase as a "marker" enzyme. Recently, it has become possible to demonstrate other lysosomal acid hydrolases by histochemical techniques.

Reported comparative histochemical studies concerning tissue and species distributions of lysosomal acid hydrolases have been very limited and fragmentary. A synopsis of the pertinent histochemical literature on distributions of lysosomal acid hydrolases in different tissues and species is presented in Appendix II, and denotes considerable heterogeneity amongst lysosomes.

Two reviews on the comparative histochemistry of acid hydrolases have appeared recently (Gahan, 1967, and Woessner, 1965). In his treatment of this topic, Woessner (1965) has abstracted from published reports semi-qualitative data on the histochemical distribution of acid phosphatase and B-glucuronidase, especially in connective tissues of various vertebrate species. Assessment of this data, however, is very difficult, because very few reported histochemical techniques, enzyme substrates, and methods of tissue preparation employed by various authors are similar.

Hayashi (1967), employed Naphthol AS-BI- compounds as substrates, and hexazonium pararosanilin as the capture reagent in simultaneous coupling, azo-dye techniques, to compare acid phosphatase, B-glucuronidase, and N-acetyl-B-glucosaminidase in terms of intensities of staining and localization in a large number of rat tissues. The foregoing enzymes were found to be widely distributed in rat tissues, acid phosphatase possessing the least apparent specialized distribution. On the other hand, B-glucuronidase and N-acetyl-B-glucosaminidase varied considerably between different tissues.

Qualitative differences in localization of the three lysosomal enzymes implied functional differences among lysosomes of different tissues.

Probable Heterogeneity of Lysosomes And Lysosomal Enzymes.

Evidence has been presented thus far for diversity amongst lysosomes, not only with respect to the dynamic changes that occur in these organelles during their life cycle within cells, but also in terms of their size, population density, and content of acid hydrolases, in different tissues of the same species, and in homologous tissues of different species.

Precise cytochemical methods are currently available for a number of well established lysosomal acid hydrolases. These provide a means of direct morphologic localization of enzyme activity to lysosomes, permit a rapid survey of patterns of enzyme activity and distribution in tissues with mixed cell populations, and provide quantitative data on the number, size, location, and approximate enzyme content of these organelles, in different tissues, and in homologous tissues in different species.

In order to partially define the limits of heterogeneity of lysosomes in different tissues of a species, and in homologous tissues of different vertebrate species, a battery of six precise cytochemical methods for acid phosphatase, B-glucuronidase, N-acetyl-B-glucosaminidase, and non-specific esterases will be applied to six standard tissues from twenty-seven vertebrate species, with representatives from Mammalia, Reptilia, Aves, Amphibia, and Pisces.

This approach is intended to provide information concerning the species distribution of lysosomes, their enzyme content, and indirect evidence concerning possible functions in diverse

locations. The foregoing will also test the validity of the use of acid phosphatase as a lysosomal "marker" enzyme.

It is also apparent from the literature reviewed that quantitative biochemical variations in the profile of lysosomal hydrolases contained within a given tissue may, in actual fact, reflect variations in size, number, and specific enzyme content of lysosomes. Biochemical results, therefore, represent mean values, often without regard to functional state or specific cell type of origin. More precise morphologic correlations can be provided by cytochemical techniques. In this context, a number of experiments also will be described in which deliberate attempts are made to produce either activation or inhibition of specific acid hydrolases of lysosomes in rat or mouse liver or kidney by :- (A) hormonal induction of lysosomal enzyme protein, (B) pinocytotic induction of lysosomes, (C) induction of cytolysosomes, (E) selective drug-inhibition of acid phosphatase activity in liver and kidney, (F) production of tissue necrosis, (G) administration of cortisone to stabilize lysosomal membranes, and (H) drug-induced formation of "residual bodies".

SECTION II

MATERIALS AND METHODS

(A) Vertebrate Species

The vertebrate species employed in this study are tabulated below: -

Phylum	Class	Genus and/or Species	Common Name	Source
Chordata	Osteichthyes (Pisces)	Stizostedion vitreum	Walleye pike	Zoology Dept.
		Esox lucius	Northern Pike	Zoology Dept.
	Amphibia	Trituru viridescens		
		Aquatic phase -	Red spoted newt	GBS
		Terrestial phase -	Salamander	GBS
		Rana pipiens	Grass frog	GBS
		Hyla versicolor	Tree frog	GBS
	Reptilia	Pseudemys elegans	Turtle	GBS
		Anolis sp.	Chamelion	GBS
	Aves	Gallus gallus	Chicken	Prov. Vet. Lab.
		Pica pica	Magpie	Dr. C. Hampson
		Turdus migratorius	Robin	Dr. C. Hampson
		Bubo virginianus	Horned owl	Dr. C. Hampson
		Cotornix sp.	Japanese quail	Zoology

Continued.

Phylum	Class	Genus and/or Species	Common Name	Source
	Mammalia	Oryctolagus cuniculus	Rabbit	Dept. of Pathology
		Cavia porcellus	Guinea pig	Dept. of Pathology
		Mesocricetus avratus	Golden Hamster	Dept. of Pathology
		Mus museulus	Mouse	Dept. of Pathology
		Rattus rattus	Rat	Dept. of Pathology
		Bos taurus	Cow	Abatoire
		Ovis sp.	Sheep	Abatoire
		Equus caballus	Horse	Abatoire
		Sus sp.	Pig	Abatoire
		Felis catus	Cat	SMRI
		Canis familiaris	Dog	SMRI
		Homo sapiens	Man	UAH

Legend:

GBS - General Biological Supply House, Inc.
8200 South Hoyne Avenue, Chicago, Illinois, 60620

SMRI - Surgical - Medical Research Institute, University of Alberta

UAH - University of Alberta Hospital morgue

(B) Animal Tissues

Six standard, homologous tissues (i.e., kidney, liver, duodenum, pancreas, spleen, and mid-cerebrum of brain) were sampled from each of the 24 species employed in the study. Adult animals of either sex were used in most instances. However, in the case of exotic species, it was sometimes necessary to utilize adolescent individuals because of difficulties of procurement and laboratory maintenance. All animals were in apparent good health, and free of natural and experimentally induced disease.

(C) Tissue Preparation

1. Sacrifice of Animals

In the case of laboratory animals and small domestic species, sacrifice was carried out by exsanguination under ether or Nembutal anaesthesia. With large domestic species, animals were sacrificed by routine abatoire practice, (i.e., stunning and exsanguination). Human tissues were obtained from routine autopsies conducted at the University of Alberta Hospital morgue. The post-mortem interval was always shorter than three hours.

2. Tissue Fixation

Small, freshly excised blocks of tissue 0.5 x 0.5 x 0.3 cm. were fixed by immersion in 4% formaldehyde with 1% calcium chloride, (Baker, 1944) at 4° C. for 6 to 12 hours. Following fixation, tissue blocks were rinsed in several changes of cold, distilled water, and stored in Holt's gum-sucrose medium (1% Gum acacia in 0.88 M sucrose) (Holt, et al, 1960) at 4° C. for 1 to 5 days.

(C) Tissue Preparation (Continued)

3. Preparation of Cryostat Sections

Blocks of fixed tissue of uniform dimensions from each of the six organs previously enumerated, were placed in a large (size 00) gelatin capsule containing "Lab-Tek" * medium (Temperature Zone 1; 0° to -15° C.), in sequential order (i.e., liver, kidney, spleen, intestine, pancreas, and brain) and frozen rapidly by immersion in 2-methyl butane chilled to -110° C. with liquid nitrogen, stored in a Dewar flask. These composite blocks of frozen tissues enclosed in a gelatin capsule were transferred to the chamber of a Harris cryostat, trimmed, and mounted lengthwise by freezing, on a cold two-inch, microtome chuck, with "Lab-Tek" O.C.T. medium. Cryostat sections of these composite tissue blocks were cut at 6 to 8 microns on a Lipshaw, rust-proof, rotary microtome equipped with an anti-roll bar, and mounted by thawing on gelatinized cover glasses. Mounted sections were stored in Columbia jars, removed from the Cryostat, rapidly dried in a stream of compressed air, and immersed in cold 4% formaldehyde with 1% calcium chloride, at 4° C. (to promote adherence of the sections to the cover glasses). This specially devised technique for preparing composite multi-tissue sections permitted the production of large numbers of flat, defect-free sections for semi-quantitative, comparative enzyme cytochemistry. Tissue sections were routinely rinsed in distilled water prior to their use in histochemical procedures.

*Address: "Lab-Tek", Westmount, Illinois, (supplied by Canadian Laboratory Supplies Ltd., Edmonton, Alberta).

(D) Cytochemical Techniques

Six cytochemical methods were routinely applied to all tissues for the demonstration of four lysosomal hydrolases.

1. Acid Phosphatase

(a) Gomori's Lead Phosphate Method (Lillie's modification, 1954). Two stock solutions were prepared in advance and stored at 4° C. These solutions were:-

(i) 0.1 M sodium B-glycerophosphate solution (3%).

(ii) 0.05 M acetate buffer, pH 5.0, containing 1.2 gm. lead nitrate per liter of solution.

The working solution was prepared by mixing one part B-glycerophosphate solution with ten parts of the lead nitrate in acetate buffer. This mixture was heated gently to 60° C., filtered, and cooled prior to use. Mounted sections were incubated at 37° C. for 30 to 45 minutes, rinsed well in distilled water, immersed in a 1:40 dilution of yellow ammonium sulfide, washed in distilled water, and mounted in Burstone's P.V.P.-glycerine medium. (Burstone, 1957).

(b) Method of Barka and Anderson utilizing Naphthol-AS-TR-phosphate and hexazonium pararosanilin. (Barka and Anderson, 1963). Stock solutions were prepared in advance and stored at 4° C. These solutions were:-

(i) A dye stock solution of hexazonium pararosanilin was prepared by dissolving 1 gm. of pararosanilin - HCl in 20 ml. distilled water and 5 ml. concentrated HCl, with gentle warming. The mixture was then cooled and filtered.

(D) Cytochemical Techniques (Continued)

(ii) 4% sodium nitrite in distilled water.

(iii) Michaelis veronal-acetate stock solution. (9.714 gm. sod. acetate. 3 H_2O + 14.714 gm. sodium barbiturate in carbon dioxide-free distilled water, made up to final volume of 500 ml.) The working solution was prepared as follows:-

10 mg. of the Naphthol-AS-TR-phosphate substrate was dissolved in a few drops of N,N,-dimethylformamide, and rapidly mixed with 5 ml. of the Michaelis veronal-acetate stock solution, diluted with 12 ml. distilled water. 0.8 ml. of each of hexazonium pararosanilin and 4% sodium nitrite solution were mixed, allowed to stand for one minute, until diazotization was complete and then added to the buffered substrate solution. The pH of the solution was then adjusted to 5.0 with 1 N NaOH, and filtered. Sections were then incubated at 37° C. for 30 to 45 minutes, washed in distilled water, and mounted in Burstone's P.V.P.-glycerine medium.

2. B-Glucuronidase

The method of Hayashi (Hayashi, 1964) for the demonstration of B-glucuronidase employing Naphthol AS-BI-glucuronide sodium salt and hexazonium pararosanilin was employed.

Stock solutions were prepared in advance and stored at 4° C.

2. B-Glucuronidase (Continued)

(a) Stock substrate solution was prepared by dissolving completely 28 mg. Naphthol-AS-BI-glucuronide (free acid) in 1.2 ml. of 0.05 M sodium bicarbonate (420 mg. $NaHCO_3$ in 100 ml. distilled water) and 0.2 N Acetate buffer, pH 5.0, was added to bring the volume to 100 ml.

(b) Hexazonium pararosanilin stock solution (see method of Barka and Anderson for acid phosphatase).

(c) 4% sodium nitrite stock solution.

The working solution was prepared just prior to use by mixing 0.3 ml. of hexazonium pararosanilin and of 4% sodium nitrite solution in a 20 ml. beaker. One minute later 10 ml. of the substrate stock solution was poured into the dye mixture, the pH was adjusted to 5.2 with 1 N NaOH. Distilled water was added to bring the final volume to 20 ml. The solution was filtered and used immediately. Sections were incubated at 37° C. for 30 to 45 minutes, washed in distilled water, and mounted in Burstone's P.V.P.-glycerine medium.

3. B-Glucosaminidase

The method of Hayashi (Hayashi, 1965) for the demonstration of B-glucosaminidase employing Naphthol-AS-BI-N-acetyl B-glucosaminide and hexazonium pararosanilin was used. The stock solutions were prepared in advance and stored at 4° C.:-

3. B-Glucosaminidase (Continued)

Hexazonium pararosanilin (see method of Barka and Anderson for Acid Phosphatase).

The working solution was prepared just prior to use by mixing 0.3 ml. of hexazonium pararosanilin and of 4% sodium nitrite solution. Diazotization was allowed to proceed for one minute. Then the substrate solution, consisting of 3 mg. Naphthol AS-BI-N-acetyl B-glucosaminide in 0.5 ml. of ethylene glycol monoethyl ether, and 5 ml. of 0.1 M citrate buffer, pH 5.2, was added. The pH of the solution was then adjusted to 5.2 with 1 N NaOH and distilled water was added to bring the final volume to 10 ml. This working solution was filtered and used immediately. Incubation of sections was carried out at 37° C. for 30 to 45 minutes. Sections were then washed in distilled water and mounted in Burstone's P.V.P.-glycerine medium.

4. Non-Specific Esterase

(a) A modification of the α-Naphthyl acetate method for the demonstration of non-specific esterase, was used (Nachlas, 1957). A stock solution of Michaelis veronal-acetate buffer was prepared in advance, and stored at 4° C. The working solution consisted of a 2% w/v solution of α-Naphthyl acetate in acetone. Michaelis veronal acetate buffer 0.1 M, pH 8.5 was then prepared from 5 ml. of stock buffer solution, 17 ml. of water and 1 ml. of 0.1 N HCl. 0.13 ml. of the substrate solution was then introduced into 10 ml. of the

4. Non-Specific Esterase (Continued)

buffer by submerging the tip of a pipette under the surface of the liquid before releasing the contents of the pipette. 15 ml. of distilled water was then added to the buffer-substrate solution, followed by 100 mg. Fast Blue BB salt. The mixture was stirred, filtered, and used immediately. Sections were incubated at 37° C. for 5 to 10 minutes, washed in distilled water, and mounted in Burstone's P.V.P.-glycerine medium.

(b) A modification of the method employing Naphthol-AS-acetate for the demonstration of non-specific esterases was used (Pearse, 1954). 10 ml. of 5% propylene glycol in 0.05 M phosphate buffer, pH 7.4, was prepared. 5-10 mg. Naphthol-AS-acetate was dissolved in 0.1 ml. acetone and then rapidly mixed with the propylene glycol-buffer mixture in order to avoid the formation of a precipitate. Approximately 15 to 20 mg. of Fast Blue BB salt was added. The resulting mixture was filtered and used immediately. Sections were incubated for 15 minutes at 37° C., washed in distilled water, and mounted in Burstone's P.V.P.-glycerine medium.

(E) MODULATIONS OF LYSOSOMAL ENZYMES BY EXPERIMENTAL MEANS

I. Induction of Lysosomal Enzymes:-

(A) Hormone Induction of a Lysosomal Enzyme

1. Administration of testosterone propionate to C_3H mice has been reported by Fishman, Artenstein and Green

(A) Hormone Induction of a Lysosomal Enzyme (Continued)

(1955) to produce a 10- to 60- fold increase in renal glucuronidase activity. Accordingly, adult male A/Jax mice were injected subcutaneously in the neck region, on alternate days, with 1 mg. of testosterone propionate dissolved in 0.1 ml. olive oil. Sacrifice was carried out on the fifteenth day, and tissues were preserved in 4% formaldehyde with 1% calcium chloride for enzyme cytochemistry. Treated control animals received 0.1 ml. olive oil only subcutaneously in the neck region on alternate days. Untreated control animals were maintained on the standard laboratory diet, ad libitum, and received no other treatment.

2. Effect of Oestrone on Glucuronidase Activity in Mouse Liver

Administration of oestrone to normal and castrate male mice has been reported by Kerr, Campbell, and Levvy (Kerr, 1949) to cause a marked rise in the glucuronidase activity of liver and uterus. Accordingly, adult A/Jax male mice received a single subcutaneous injection in the neck region of 0.05 mg. estrone, dissolved in 0.25 ml. of olive oil. Sacrifice was carried out three days later. Tissues were fixed in formol-calcium for enzyme histochemistry. Untreated and treated control animals were respectively maintained on a normal diet, or injected subcutaneously with olive oil only.

(B) Induction of Lysosomal Enzymes Through Formation of Neolysosomes

1. Agents that Induce Pinocytosis

Dextran, M.W., 160,000, (50 mg. in 1 ml. 0.9% sodium chloride solution) was administered intraperitoneally to A/Jax albino male mice, on nine successive days according to the method reported by Meijer, et al., (1961). Control rats received 0.9% sodium chloride solution only, intraperitoneally, and negative control rats received no injections. Animals were sacrificed on the 10th day and tissues were processed for study using the battery of cytochemical tests described previously.

2. Production of Cytolysosomes by the Administration of Glucagon

Intraperitoneal administration of glucagon to rats (50 micrograms per 100 grams body weight), has been reported by Deter and de Duve (Deter, 1967) to rapidly induce the formation of cytolysosomes in liver. Accordingly, 200 g. male, Wistar rats received intraperitoneal injections of 50 micrograms per 100 gms. body weight of glucagon suspended in 0.2 M glycine-sodium hydroxide buffer, pH9.5. Treated animals were sacrificed 90 minutes later. Blocks of liver and kidney were fixed in cold 4% formaldehyde with 1% calcium chloride for enzyme cytochemistry, and in Caulfield's Osmium tetroxide for electron microscopy.

II. INHIBITION OF A LYSOSOMAL ENZYME

Acid Phosphatase

Shibko and Friedman (Shibko, 1966) have reported that oral administration of N-2-fluorenylacetamide produces a pronounced selective depression of acid phosphatase activity in rat liver and kidney, but not in other organs, eight to sixteen hours after administration. Accordingly, 100 mg. of N-2-fluorenylacetamide suspended in 1 ml. olive oil, was administered by stomach tube as a single dose to 200 gm. male Wistar rats. Experimental animals were sacrificed sixteen hours later, and representative tissues were collected into formol-calcium for fixation for enzyme histochemistry.

III. EFFECT OF DRUGS ON HISTOCHEMICALLY DEMONSTRABLE LYSOSOMAL HYDROLASES

(A) Cortisone

Cortisone in high dosage has been reported to stabilize lysosomal membranes (Weissmann and Dingle, 1962). Cortisone, in dosage of 10 mgm., g.d., was administered subcutaneously for 10 days, to adult male Wistar rats, weighing 250 gms. Animals were sacrificed by exsanguination under ether anesthesia and liver and kidney tissues were fixed in formol-calcium for enzyme histochemistry.

(B) Phenacetin

Phenacetin has been reported to induce the formation of residual bodies with histochemical properties of lipofuscin in rat kidney under conditions of long term

III. Continued.

administration. Male Wistar rats weighing 200 gms. were maintained for nine weeks on a powdered diet that contained 1.8% phenacetin. Blocks of liver and kidney were fixed in formol-calcium for enzyme histochemistry.

IV. EFFECT OF PHOSPHORUS-INDUCED HEPATOCELLULAR NECROSIS ON HISTOCHEMICALLY DEMONSTRABLE LYSOSOMAL HYDROLASES

de Duve (de Duve, 1959) has suggested that lysosomal hydrolases may play a role in the production of necrosis. Peripheral zonal hepatic necrosis was induced experimentally in 200 gm. male Wistar rats, by the administration by stomach tube of a suspension of 1.2 mg. of yellow phosphorus in 2 ml. of mineral oil. Experimental animals were sacrificed 18 hours later and blocks of liver were taken for fixation in formol-calcium for enzyme histochemistry.

MODULATIONS OF LYSOSOMAL ENZYMES BY EXPERIMENTAL MEANS

(METHOD)	(REFERENCE)
I. INDUCTION:-	
A. Hormone Induction of a Lysosomal Enzyme	
(1) Effect of Testosterone propionate on glucuronidase in mouse kidney	Fishman,W.H., Artenstein, M., and Green, S., Endocrinology, 57: 646, 1955.
(2) Effect of Oestrone on glucuronidase in mouse liver	Kerr, L.M.H., Capbell, J.G., Levvy, G.A., Biochem. J., 44: 487, 1949.
B. Through Formation of New Lysosomes	
(1) By agents that induce pinocytosis (i.e., dextran)	Meijer, A.E.F.H., and Willighagen, R.G.J., Biochem. Pharmacology, 8: 389, 1961.
(2) By production of cytolysosomes by the administration of glucagon	Deter, R.L., and de Duve, C., Journal of Cell Biology, 33: 437, 1967.
II INHIBITION:-	
Inhibition of a Lysosomal enzyme (Acid phosphatase) in liver and kidney by the administration of N-2-fluorenylacetamide	Shibko, S., Toxicology and Applied Pharmacology, 10: 548,1967.

MODULATIONS OF LYSOSOMAL ENZYMES BY EXPERIMENTAL MEANS (COntinued)

(METHOD)	(REFERENCE)
III. EFFECTS OF DRUGS ON HISTOCHEMICALLY DEMONSTRABLE LYSOSOMAL HYDROLASES	
A. Cortisone	Tsutsumi, S., et al., Jap. J. Pharmacol. 16: 443, 1966.
B. Phenacetin	Torhorst, J., Rohr, H.P., Zollinger, H.U., Studer, A., and Trazer, J.P., Virch. Arch. Path. Anat., 342:70, 1967.
IV. EFFECT OF PHOSPHORUS-INDUCED HEPATOCELLULAR NECROSIS ON HISTOCHEMICALLY DEMONSTRABLE LYSOSOMAL ACID HYDROLASES	Barker, E.A., et al., J. Lab. Invest. 12: 955, 1963.

III. RESULTS

Histochemistry of Lysosomal Acid Hydrolases

I. MAMMALS

A. Kidney:

The basic structural and functional unit of the metanephric mammalian kidney is the nephron. The constituents of this unit in sequential order are: the Malpighian corpuscle surrounded by Bowman's capsule, a short constricted neck piece, the proximal convoluted tubule, loop of Henle with its descending thin and thick limbs, followed by the collecting tubule, and the collecting duct. The latter empty into the renal pelvis. The loops of Henle are usually short in man, but are predominantly long in the ox and pig and exclusively long in the cat. Similarly, the collecting tubule which is short in many mammals, is long in the horse, pig and cat.

Lysosomal acid hydrolase activity of the mammalian kidney is found principally in the cells that line the proximal tubules and in the intercalated cells of the collecting tubules.

(1) Proximal tubules

(a) Acid phosphatase activity

Acid phosphatase activity (B-glycerophosphate phosphatase and Naphthol AS-TR-phosphate phosphatase) is particularly high in the majority of the mammals investigated (rat, Fig. 5; dog, Fig. 7; cow; sheep; horse, Fig. 10; mouse (Ajax), Fig. 241; field mouse, Fig. 9; hamster, Fig. 8; rabbit; guinea pig, Fig. 2; pig, Fig 12). The proximal tubules of man (Fig. 22), and cat display low activity for these isoenzymes. Lysosomes

are numerous and small to medium in size in most of the mammals, except for the rat which possesses large lysosomes.

(b) Glucuronidase activity

Numerous small lysosomes with moderate to high glucuronidase activity are present in dog, Fig. 13; cat; cow; sheep; horse; mouse (Ajax), Fig. 243; and man (adult), Fig. 24. Much lower activity in fewer lysosomes is found in rat, hamster, and guinea pig. The remaining mammals (rabbit, field mouse), possess no glucuronidase activity.

(c) Glucosaminidase activity

Glucosaminidase activity is moderate to high in some mammals (dog, Fig. 25; cow; sheep, Fig. 16; horse; rat; guinea pig; pig; cat, Fig. 17). In others, this activity is low to moderate (mouse (Ajax); man (adult), Fig. 23; field mouse), or negative (rabbit).

(d) Esterase activity

a-Naphthyl acetate esterase activity is distributed diffusely in cytoplasm, and is very intense in all of the mammals examined (Figs. 18, 21, 26). Naphthol AS-acetate esterase activity of moderate to high intensity, occurs in both granular and diffuse forms in proximal tubules, in all the animals studied (Figs. 19, 20, 29, 30).

(2) Intercalated cells

(a) Acid phosphatase activity

High B-glycerophosphate phosphatase and Naphthol AS-TR-phosphate phosphatase activities are present in renal intercalated cells of some mammals (rat, hamster, rabbit, horse, pig, cow), while others (sheep, mouse (Ajax), guinea pig), possess only high B-glycerophosphate

phosphatase activity. The cat possesses only Naphthol AS-TR-phosphate phosphatase activity.

(b) Glucuronidase activity

High activity for this enzyme is present in intercalated cells of the hamster; other animals possess low to moderate activities confined to small lysosomes (horse, sheep, rat, man (adult), dog, cow, guinea pig, mouse (Ajax), rabbit). Glucuronidase activity is totally absent from these cells in pig, cat, and field mouse.

(c) Glucosaminidase activity

Like glucuronidase activity of intercalated cells, most mammals possess low to moderate glucosaminidase activity. A few, however, do possess high activity for this enzyme (cat, mouse (Ajax)). Field mouse and rabbit possess no glucosaminidase activity.

(d) Esterase activity

a-Naphthyl acetate esterase activity is found to be diffuse and of low intensity in some mammalian intercalated cells (rat, hamster, guinea pig, mouse (Ajax), horse, pig, sheep, cat). In others, diffuse activity is absent and only lysosomal activity is noted (field mouse, rabbit, man (adult), cow). Only the dog is devoid of a-Naphthyl-acetate esterase activity.

Naphthol-AS-acetate esterase activity in intercalated cells is high in a few mammals (rat, hamster, man (adult), horse), low in most of the remaining mammals (guinea pig, mouse (Ajax), field mouse, rabbit, sheep, dog), and absent only in the cat.

B. Liver:

The lobule is the smallest functional unit of the mammalian liver. Collectively these constitute the parenchyma of liver. Radially arranged plates of liver cells are perfused through sinusoids that drain into a central vein. Liver macrophages, or Kupffer cells, line the sinusoidal spaces. Between adjacent liver cells, bile capillary spaces form a network that drains into intrahepatic bile ducts located in portal triads. Branches of the portal vein, hepatic artery, and bile ducts, surrounded by connective tissue, are located at the periphery of each lobule.

Lysosomal acid hydrolase activity in mammalian liver cells is found in pericanalicular regions, in Kupffer cells, and in some instances in bile duct epithelium.

(1) Parenchymal cells

(a) Acid phosphatase activity

The majority of mammals possess high lysosomal acid phosphatase (B-glycerophosphate phosphatase and Naphthol AS-TR-phosphate phosphatase) activity, confined to numerous small lysosomes. Man (adult), Fig. 28; possesses low activities for these enzymes, and sheep only possess Naphthol AS-TR-phosphate phosphatase activity. Only small numbers of lysosomes are found in hepatocytes of the cow and field mouse. Both acid phosphatase isozymes are of low activity in the lysosomes of the cow, whereas the field mouse only possesses low activity of Naphthol AS-TR-phosphate phosphatase.

(b) Glucuronidase activity

Lysosomes bearing glucuronidase activity are very small but

numerous in most of the mammals investigated. Activity is highest in rat, Fig. 41; guinea pig, Fig. 42; rabbit; sheep, Fig. 44; man (infant and adult), Fig. 38; and cow. The remaining mammals (hamster, Fig. 45; mouse (Ajax), Fig. 244; horse, Fig. 43; cat, Fig. 47; field mouse; pig; dog) possess low activity for this enzyme.

(c) Glucosaminidase activity

Glucosaminidase activity, in contrast to glucuronidase activity, is distinctly lower in most of the mammals investigated. Guinea pig is the only animal with high glucosaminidase activity in hepatocytes. Of the other mammals, some (horse; pig; cat; man (adult and infant); dog, Fig.39) display low activity for this enzyme, and some (rat, hamster, mouse (Ajax), field mouse, rabbit cow, sheep), are devoid of glucosaminidase activity.

(d) Esterase activity

High, granular and diffuse esterase activity is present in cytoplasm of parenchymal cells of all mammals except man (infant), Fig. 40; and field mouse, where activities are low.

(2) Kupffer cells

(a) Acid phosphatase activity

Acid phosphatase activity is moderate to very high in lysosomes of Kupffer cells of most mammals investigated. Differences in the numbers and sizes of lysosomes exist, however, between different mammals. The majority of mammals (man (infant, Fig. 27 and adult, Fig. 28); rat, Fig. 34; mouse (Ajax), Fig. 242; pig; cow), possess numerous lysosomes, while relatively few lysosomes are found in some of the remaining mammals studied (rabbit; horse, Fig. 33; hamster, Fig. 35; sheep; cat,

Fig. 36). Guinea pig (Fig. 32), dog, and field mouse, possess numerous small lysosomes with high Naphthol AS-TR-phosphate phosphatase activity. High activity of B-glycerophosphate phosphatase, on the other hand, is found in only few lysosomes in these animals.

(b) Glucuronidase activity

Precisely localized, high lysosomal glucuronidase activity, is present in most mammalian Kupffer cells. However, differences in the numbers of lysosomes in this group of mammals do exist; - i.e., these organelles are numerous in rat, Fig. 41; sheep, Fig. 44; horse, Fig. 43; dog; and man (infant), Fig. 38, and are few in hamster, Fig. 45; rabbit; cat, Fig. 47; guinea pig, Fig. 42; and field mouse. Low glucuronidase activity is found in cow, pig, and man (adult), and no activity is present in mouse (Ajax) Kupffer cell lysosomes.

(c) Glucosaminidase activity

Lysosomes are numerous in field mouse, cow, sheep, and man (infant and adult) and are few in the remaining mammals investigated. Moderate to high activity is present in cow; sheep; cat; dog, Fig. 39; pig; and guinea pig, and low to moderate activity is present in field mouse, man (infant and adult), rat, hamster, rabbit and horse.

(d) Esterase activity

a-Naphthyl acetate and Naphthol AS-acetate esterase activities are high in most mammals examined. The former isoenzyme activity in dog, and the latter isoenzyme activity in man (infant) and dog, are present in small but numerous lysosomes, while both activities in the remaining mammals appear diffusely in the cytoplasm (Figs. 40, 46, 48).

(3) Bile duct epithelium

Acid phosphatase activity is low in bile duct epithelium. Glucuronidase activity is high in guinea pig and dog, and is low in rat, mouse (Ajax) and man (infant). Glucuronidase activity is absent in field mouse, rabbit, horse, pig, cow, hamster, sheep and cat.

Glucosaminidase activity is high in guinea pig, dog, and man (infant). The activity is low in man (adult), hamster, sheep and cat. No glucosaminidase activity is found in field mouse, rabbit, horse, pig, cow, rat, and mouse (Ajax). a-Naphthyl acetate esterase of high activity is present only in mouse (Ajax). Hamster, field mouse, cow, and sheep are devoid of a-Naphthyl acetate esterase activity. Other animals not listed show low activity at this site.

Activity of Naphthol AS-acetate esterase in bile duct epithelium is high in rabbit and horse. The remaining mammals possess very low activity.

C. Pancreas:

The mammalian pancreas is a lobulated, compound, tubuloacinous gland composed of exocrine and endocrine components. The exocrine portion of each lobule consists of numerous acini lined by secretory cells which discharge into acinar ducts. These ducts unite into intralobular intercalated ducts which in turn form interlobular ducts. The latter anastomose to form the extrapancreatic duct, which links the organ with the duodenum.

The endocrine component, the Islets of Langerhans, is composed of a network of cellular cords interspersed with sinusoidal capillaries. Islets of Langerhans are scattered throughout the lobules of the exocrine pancreatic tissue and are most numerous in the tail of the pancreas.

(1) Acinar cells

Man (adult and infant), Fig. 59; with high activity, and horse and hamster with moderate activity, are the only mammals with B-glycerophosphate phosphatase activity. Naphthol AS-TR-phosphate phosphatase activity is high only in man (infant and adult), Fig. 60. The remaining mammals possess low Naphthol AS-TR-phosphate phosphatase activity. Moderate glucuronidase activity is present only in man (infant). Glucosaminidase activity is almost absent from the acinar cells of all the mammals examined (Fig. 63). a-Naphthyl acetate esterase activity represented by diffuse and granular deposits of reaction product, is moderate to high in some mammals (man (adult and infant), Fig. 61; dog, Fig. 62; horse), and low in others (hamster; sheep; guinea pig; mouse (Ajax); field mouse; rabbit; cow; cat, Fig. 66). Only man (infant, Fig. 64 and adult) possesses high Naphthol AS-acetate esterase activity, while the remaining mammals have low to moderate activities (Fig. 67).

(2) Islet cells

Most mammals possess low lysosomal acid phosphatase activity, and some (horse, cow, sheep) are negative for B-glycerophosphate phosphatase activity. Islet cell glucuronidase activity, on the other hand, is low in few mammals (rat, mouse (Ajax), cat) and is absent from the majority of mammals. Similarly, islet cell glucosaminidase activity is very low or absent. Esterase activity of islet cells is very low in all mammals studied (Figs. 61, 64, 67).

(3) Pancreatic duct epithelium

Acid phosphatase activity of epithelium of pancreatic ducts for

Naphthol AS-TR-phosphate and B-glycerophosphate isozymes is low in hamster; guinea pig; mouse (Ajax); cow; dog; man (infant, Fig. 59 and adult) and horse. Rat and pig are deficient in B-glycerophosphate phosphatase activity, while the horse is deficient in Naphthol AS-TR-phosphate phosphatase. Acid phosphatase activity is lacking in field mouse, rabbit, sheep, and cat.

Glucosaminidase and glucuronidase activities are low or absent in the pancreatic duct epithelium of most of the mammals studied (Fig. 63).

Man (infant and adult), mouse (Ajax), and cat possess high a-Naphthyl acetate esterase activity, while this activity is low in rabbit, horse, and dog, and is totally absent from rat, hamster, guinea pig, field mouse, pig, cow, and sheep. Moderate to high activities for Naphthol AS-acetate esterase are present in some mammals (man (infant and adult), guinea pig, rabbit, horse, pig, cow), but low activities are found in the remainder of the mammals investigated.

D. Spleen:

The mammalian spleen is composed of a parenchyma consisting of two types of pulp: white pulp made up of numerous splenic corpuscles with germinal centres and a surrounding cuff of small lymphocytes, and red pulp, interspersed between splenic corpuscles, characterized by the presence of arteries emerging from the splenic corpuscles, sinusoids, and cellular elements of the blood. Sinusoids are lined by numerous macrophages. Reticular tissue supports both the red and white pulps.

Lysosomal acid hydrolase activity of the spleen is primarly localized to the macrophages of the red and white pulps.

(1) Macrophages of the red pulp

(a) Acid phosphatase activity

Most mammals investigated possessed high enzyme activity confined to numerous small to medium size lysosomes (Figs. 49, 50, 51, 53, 56, 58). A few, however, are not endowed with this high activity, (man (adult), Fig. 57; cat). The hamster is negative for B-glycerophosphate phosphatase activity.

(b) Glucuronidase activity

Glucuronidase activity is high in all mammalian red pulp macrophages (Fig. 52). Differences in the number of lysosomes do exist, however, between different mammals. Such lysosomes are numerous in rat; hamster; guinea pig, Fig. 54; mouse (Ajax); field mouse; cow; and man (infant) and are few in horse, pig, sheep, cat and man (adult).

(c) Glucosaminidase activity

A few mammals (hamster, guinea pig, field mouse, horse), possess very high glucosaminidase activity. Some possess only low activity (pig, mouse (Ajax), cow, sheep, man (infant), cat), and a few possess no activity (man (adult), rabbit).

(d) Esterase activity

Moderate to high activity for a-Naphthyl acetate esterase activity is found only in field mouse and mouse (Ajax). Low to moderate esterase activity is found in the remaining mammals. Variations in numbers and sizes of lysosomes do exist however. In contrast to the foregoing isoenzyme, Naphthol AS-acetate esterase activity is very intense in rat; hamster; guinea pig; mouse (Ajax); field mouse, Fig. 55;

and cow. Activities in the remaining mammals are moderate (rabbit, horse, pig, cat) or absent (sheep).

(2) Cords of Billroth

Cells of the cords of Billroth possess very low or absent activities for lysosomal acid hydrolases.

(3) Macrophages of the white pulp

(a) Acid phosphatase activity

Acid phosphatase activity is localized to small and medium size lysosomes in most mammals. The activity is relatively high in rat, Fig. 50; hamster; mouse (Ajax); field mouse, Fig. 53; horse, Fig. 56; and cow. Some mammals possess high activity for only B-glycerophosphate phosphatase (rabbit, pig) and some possess high activity for only Naphthol AS-TR-phosphate phosphatase (man (infant), guinea pig, cow). Acid phosphatase activities are low in the cat and man (adult), Fig. 57; while sheep and pig, Fig. 49; possess low activities for only Naphthol AS-TR phosphate phosphatase. B-glycerophosphate phosphatase activity is negative in sheep and Naphthol AS-TR phosphate phosphatase is absent from rabbit white pulp macrophages.

(b) Glucuronidase and glucosaminidase activities

In most mammals, possessing these enzyme activities, lysosomes are small and medium in size. High activities for both enzymes are found in rat; hamster; guinea pig, Fig. 54; mouse (Ajax) and cat. Field mouse possesses high activity for only glucosaminidase. Activities of both enzymes are low in cow, while glucuronidase activity only, is low to moderate in field mouse; rabbit, Fig. 52; man (adult) and horse. The sheep possesses neither enzyme activity, and glucosaminidase activity

is absent from rabbit, horse, and man (adult).

(c) Esterase activity

The horse possesses high a-Naphthyl acetate esterase activity. Most of the remaining species possess low activity for this isozyme. The sheep is found to be negative.

The most intense Naphthol AS-acetate esterase activity is present in hamster, horse, and cow. Slightly lower activity is found in guinea pig; mouse (Ajax); field mouse, Fig. 55; and rabbit. Quite low activities, on the other hand, are found in the remainder of animals examined (sheep, cat, man (adult and infant), pig, rat).

Lymphoid cells in white pulp do not possess acid hydrolase activity.

E. Duodenum:

The duodenum, is the first portion of the small intestine. Its functions are (1) the propulsion of food, (2) the digestion of the chyme by digestive juices, and (3) the absorption of nutritive material.

To facilitate these functions the surface area of the duodenum is greatly increased by the presence of circular valves of Kerckring and countless microscopic filli (0.5 - 1.5 mm. long) on the mucous surface. Crypts of Lieberkühn open between the bases of the villi.

The epithelium of the mucous membrane, is of simple columnar type and consists of three kinds of cells, (a) columnar cells with striated borders, (b) goblet cells, and (c) argentaffin cells. A thin basement membrane supports these cells, and it in turn rests on the lamina propria of the mucous membrane. The latter is made up of argyrophil fibres, blood vessels, lymphatic channels, macrophages,

eosinophils, and small lymphocytes.

Lysosomal acid hydrolase activity in duodenum occurs principally in the villous epithelial cells, (apical, middle, and crypt) and lamina propria macrophages.

(1) Epithelial cells

(a) Acid phosphatase activity

Rat; hamster, Fig. 73; guinea pig, Fig. 68; pig, Fig. 71; and cow, possess moderate to high acid phosphatase activity in apical cells. The rabbit possesses moderate B-glycerophosphate phosphatase activity while horse, Fig. 70; and mouse (Ajax), possess moderate Naphthol AS-TR-phosphate phosphatase activity. Low acid phosphatase activity is present in apical duodenal epithelial cells of man (infant), cat, and dog, while mouse (Ajax) possesses low B-glycerophosphate phosphatase activity, and field mouse and rabbit display low Naphthol AS-TR-phosphate phosphatase activity. Man (adult) is devoid of the latter enzyme.

(b) Glucuronidase activity

Glucuronidase activity is present in mouse (Ajax); field mouse; horse; hamster, Fig. 75; guinea pig; rabbit. The remaining mammals examined (pig; cow; man (infant and adult); rat, Fig. 72; cat; dog), are without glucuronidase activity in apical epithelium.

(c) Glucosaminidase activity

Hamster; guinea pig, Fig. 74; and cat, Fig. 76, possess moderate activities for glucosaminidase and the majority of the remaining mammals (mouse (Ajax), field mouse, horse, rat, dog), possess low activity for this enzyme. Rabbit is the only mammal without glucosaminidase activity.

(d) Esterase activity

Diffuse, but high, a-Naphthyl acetate esterase activity is present in rat, hamster, guinea pig, horse, pig, cow, sheep, and man (infant). Granular deposits of reaction product are present also in mouse (Ajax), field mouse and rabbit. The dog possesses low activity while the cat is negative for this enzyme activity.

Naphthol AS-acetate esterase activity occurs diffusely in the cytoplasm. It is moderate to high, in most mammals (hamster; guinea pig; mouse (Ajax); field mouse; rabbit; horse; pig, Fig. 69; man (infant); cow), and low to moderate in rat, sheep, cat and dog.

In general, lysosomal acid hydrolase activities decrease in intensity from apical epithelial cells to crypt epithelial cells.

(2) Macrophages of the lamina propria

(a) Acid phosphatase activity

The lysosomes displaying acid phosphatase activity in all the animals studied are small to medium in size. High activities of both B-glycerophosphate phosphatase and Naphthol AS-TR-phosphate phosphatase, in numerous such lysosomes, are seen in rat; hamster, Fig. 73; guinea pig, Fig. 68; pig; cow; and field mouse. Fewer lysosomes with similar activity are present in mouse (Ajax). Cat; dog; and horse, Fig. 70; display high Naphthol AS-TR-phosphate phosphatase activity but B-glycerophosphate phosphatase activity is low in the dog and negative in the cat. Man (infant) possesses low acid phosphatase activity and man (adult) is devoid of Naphthol-AS-TR-phosphate phosphatase activity in this site.

(b) Glucuronidase activity

Most of the mammals possess moderate to high glucuronidase activity in small to medium size lysosomes which are numerous in rat, Fig. 72; guinea pig; mouse (Ajax); cow; and cat. Lysosomes are few in hamster, Fig. 75; field mouse; rabbit; horse; pig; and dog. Man (adult and infant) possess only low activity for this enzyme.

(c) Glucosaminidase activity

Glucosaminidase activity is high in guinea pig, Fig. 74; field mouse; horse; cow; rat; hamster; pig; cat, Fig. 76; and dog. Very low activity only is found in mouse (Ajax), man (infant and adult), and rabbit.

(d) Esterase activity

In all mammals examined, activity for Naphthol AS-Acetate esterase is present in granular form diffusely throughout the cytoplasm. Activity is high in cow; horse and field mouse, and low to moderate in mouse (Ajax); rabbit; pig, Fig. 69; man (infant); rat; hamster; sheep; cat; and dog.

a-Naphthyl acetate esterase activity of lamina propria macrophages is particularly low in most species examined.

F. Brain:

Histochemical investigation of lysosomal acid hydrolase activity in mammalian brain was limited to cerebral hemispheres and cerebellum.

The substance of cerebral hemispheres consists of an inner region of white matter enveloped by an outer layer of cortical grey matter. The outer surface of the cerebral hemispheres are greatly

corrugated, especially in man, by sulci, which separate the cortical gyri.

Microscopically the cerebral cortex exhibits six layers. The outermost, or molecular layer, consists primarily of fibrous elements of underlying cells and of a few cells. The second layer is the outer granular layer. composed of many small nerve cells. The pyramidal cell layer, so named because of the presence of pyramidal-shaped cell bodies of neurones, is the third layer. Next, lies the inner granular layer, which, like the outer granular layer, is composed of numerous small nerve cells. The fifth layer is the ganglionic or internal pyramidal layer, which is made up of numerous large pyramidal cell bodies of neurons. In the motor areas of the cortex these cells are called Betz cells. The inner most sixth layer of the cerebral cortex is called the polymorphous cell layer due to the variety of cell shapes present. The neuroglia, or supporting cells for brain tissue, are dispersed throughout all six layers of the cortex. Underlying white matter consists of myelinated nerve fibers and supporting neuroglia.

The cerebellum, like the cerebrum, is divided into lobes by transverse fissures, and the lobes, in turn, are crossed transversely by folia. The cerebellum consists of an outer cortex and an inner layer of white matter surrounding a core of grey matter. Microscopically, the cortex possesses three layers, (1) an outer layer of many non-medullated fibers but few cells, (2) an intermediate single layer of Purkinje cells, and (3) an inner granular layer consisting of bodies of small nerve cells. The white matter differs little from that found in the cerebral hemispheres.

The investigation of lysosomal acid hydrolase activities in mammalian cerebellum and cerebrum, was restricted to cortical neurones of cerebrum, Purkinje cells of cerebellum, endothelial cells of blood vessels, glial cells, and perithelial microglia cells.

(1) Cortical neurons

(a) Acid phosphatase activity

Acid phosphatase activity in these cells is confined to small lysosomes. These are numerous and possess moderate to high activity of both B-glucerophosphate phosphatase and Naphthol AS-TR-phosphate phosphatase, in hamster, Fig. 77; mouse (Ajax); and man (infant), Fig. 81. Similar activities of B-glycerophosphate phosphatase only, are present in guinea pig; rabbit, Fig. 78; and pig. The activities of the two isozymes of acid phosphatase are low to moderate in the remaining mammals investigated. B-glycerophosphate phosphatase is absent in sheep.

(b) Glucuronidase activity

Lysosomal glucuronidase activity of moderate intensity is found only in mouse (Ajax). Cat and hamster, only, possess low glucuronidase activity.

(c) Glucosaminidase activity

Guinea pig, Fig. 79, displays very high activity for this enzyme, in contrast to the other mammals which possess little or no activity.

(d) Esterase activity

In general, a-Naphthyl acetate esterase activity is moderate

or low in most mammals (hamster, mouse (Ajax), horse, cow, sheep, man (infant), cat), and negative in others (guinea pig, pig). Moderate to very high activity is present for Naphthol-AS-acetate esterase in some animals (rat, Fig. 80; cow; hamster; horse; pig), while of the remaining animals, some (man(infant), cat, mouse (Ajax), rabbit) possess low activity, and one (sheep) possesses no activity

(2) Purkinje cells

(a) Acid phosphatase activity

Mouse (Ajax), hamster, and man (infant), possess moderate to very high activity for acid phosphatase activity. Others (horse, cow, rat, guinea pig, rabbit, pig, cat), possess only low activity. Sheep are totally devoid of acid phosphatase at this site, and the horse possesses no activity for the a-Naphthyl AS-TR-phosphate phosphatase isozyme.

(b) Glucuronidase activity

Purkinje cells of all mammals studied are essentially negative for this lysosomal enzyme activity.

(c) Glucosaminidase activity

Low activity for glucosaminidase is found in the lysosomes of rat, rabbit, guinea pig, and dog. The remaining mammals examined (hamster, mouse (Ajax), horse, pig, cow, man (infant), sheep) show no activity.

(d) Esterase activity

Low a-Naphthyl-acetate esterase activity is found only in the diffuse form, while low a-Naphthyl-AS-acetate esterase activity appears in a granular form, in most of the mammals studied. The rabbit is devoid

of the former activity, whereas guinea pig, rabbit, sheep, and man (infant) are negative for the latter isoenzyme.

(3) Glial Cells

(a) Acid phosphatase activity

Acid phosphatase activity, generally, is of low to moderate intensity, and is found in small lysosomes. Some animals, however do not possess B-glycerophosphate phosphatase activity (horse, sheep) and others (sheep, rabbit, cat), do not display Naphthol AS-TR activity.

(b) Glucuronidase activity

Glial cells, are negative for glucuronidase activity in all mammals, except the cat which possesses low activity confined to few small lysosomes.

(c) Glucosaminidase activity

Rat, guinea pig, and rabbit, possess high to moderate glucosaminidase activities. The cat exhibits low activity. The remaining mammals examined (man (infant), mouse (Ajax), horse, pig, cow, sheep, hamster), possess no glucosaminidase enzyme activity.

(d) Esterase activity

Esterase enzyme activity of glial cells is low. Sheep, rat, and horse possess a-Naphthyl-acetate esterase activity, whereas, Naphthol-AS-acetate esterase activity is confined to rat,hamster, guinea pig, horse, pig, and cow.

(4) Endothelial Cells

Acid phosphatase, glucuronidase, and glucosaminidase activities,

are not present in endothelial cells of the mammals examined. Horse, cow and rat possess a-Naphthyl acetate esterase activity, while Naphthol AS-acetate esterase activity is found only in rat and cat.

(5) Perithelial microglia

(a) Acid phosphatase activity

Lysosomes are small and numerous and possess high activity in rat and hamster. Activity is low to moderate in guinea pig, mouse (Ajax), cow, rabbit, man (Infant), and pig. Moderate Naphthol AS-TR-phosphate phosphatase activity is present only in horse and sheep. Horse and sheep are negative for B-glycerophosphate phosphatase activity, and the cat is without acid phosphatase activity at this site.

(b) Glucuronidase activity

Rat and hamster lysosomes are numerous and contain moderate glucuronidase activity. Enzyme activity of equal intensity, but limited to fewer lysosomes, is found in guinea pig, mouse (Ajax), pig and cat. Man (infant), cow, and sheep, possess low glucuronidase activity, and rabbit and horse show no activity.

(c) Glucosaminidase activity

Glucosaminidase activity is high in rat, hamster, cat, mouse (Ajax), and horse, and low in pig, cow, sheep, and man (adult). The rabbit lacks this enzyme activity.

(d) Esterase activity

The rat alone possesses high, diffuse a-Naphthyl-acetate esterase activity, while in most other mammals (mouse (Ajax), man (adult), horse, pig cow, sheep, cat) this activity is moderate to high and

confined to lysosomes. The rabbit is without this isoenzyme. High lysosomal Naphthol AS-acetate esterase activity is found in rat, Fig. 80; hamster; mouse (Ajax); man (infant); horse; pig; low activity occurs in rabbit, cow, cat. The guinea pig shows no activity for this isoenzyme.

II. BIRDS

The following species of birds were surveyed histochemically for distribution of acid hydrolases in various organs:- Japanese quail magpie horned owl, robin, and domestic chicken.

A. Kidney:

The avian kidney is composed of pyramidal lobes, which correspond to the cortical lobules of the mammals. The bases of lobes lie under the capsule and apices project into the hilus of the kidney. Glomeruli are smaller, and proximal convoluted tubules are larger in the avian than in the mammalian kidney.

The avian nephron is composed of:

(a) a renal corpuscle or glomerulus almost encapsulated by a capsule, i.e., Bowman's capsule.

(b) a proximal convoluted segment.

(c) a medullary portion of the proximal convoluted segment which at the medullary loop becomes,

(d) the distal convoluted segment, which opens into,

(e) the collecting tubule.

The renal artery gives rises to interlobar arteries, which branch and form interlobular arteries from which the afferent vessels are formed to supply the glomeruli. Efferent vessels join the renal vein. Branches from the renal portal vein, on the other hand, pass to the tubules. Capillaries, about the tubules, empty into central converging intralobular veins, which in turn, empty into larger lobular veins, and thence the renal vein.

Lysosomal acid hydrolase activity in the avian kidney is located principally in proximal tubules, intercalated cells of collecting

tubules, and cells of the distally located collecting tubules.

(1) Proximal convoluted tubules

(a) Acid phosphatase activity

Acid phosphatase activity in this site is moderately abundant or abundant in all birds examined. The lysosomes are small to medium in size, and are numerous in Japanese quail (Fig. 88), horned owl (Fig. 94), chicken (Fig. 90), and robin (Fig. 89), and are few in the magpie (Figs. 85, 86).

(b) Glucuronidase and glucosaminidase activities

Activities of these two enzymes are high in Japanese quail, magpie (Fig. 87), and robin, and moderate in chicken. Horned owl (Fig. 93), in contrast, possesses only glucosaminidase activity in significant amounts, glucuronidase activity being totally absent. Glucuronidase-containing lysosomes, generally, in all the birds are small to medium in size, and are numerous only in Japanese quail and horned owl.

(c) Esterase activity

a-Naphthyl acetate esterase activity is high and diffuse throughout cytoplasm in all birds examined. In addition, the chicken also possesses granular activity.

Moderate activity of Naphthol-AS acetate esterase is found in granular and diffuse form in all birds.

(2) Intercalated cells of collecting tubules

(a) Lysosomal acid hydrolase activity

Lysosomes of intercalated cells possess moderate acid phospha-

tase activity but are small and sparse in Japanese quail, chicken and horned owl. Glucuronidase and glucosaminidase activities of low intensity are also found in these three birds. Esterase activity is absent from all intercalated cells of birds examined.

(3) Distally located collecting tubules

(a) Acid phosphatase activity

Cells in these tubules possess only a few small lysosomes. Acid phosphatase is generally low in birds (Japanese quail, magpie, robin) in this location. The horned owl exhibits only B-glycerophosphate phosphatase activity.

(b) Glucuronidase activity

Japanese quail and magpie possess low glucuronidase activity; the remaining birds are negative for this enzyme activity.

(c) Glucosaminidase activity

Robin possesses high activity for this enzyme, in contrast to low activities in Japanese quail and horned owl, and no activity in the magpie.

(d) Esterase activity

Activity of a-Naphthyl acetate esterase, in both granular and diffuse forms, is of low to moderate intensity in horned owl and robin, respectively. Japanese quail and magpie, on the other hand, are negative for this activity.

Moderate Naphthol-AS acetate esterase activity is present in robin. Japanese quail, and horned owl possess only low activity, and no activity is present in chicken and magpie for this esterase isoenzyme.

B. Liver:

The structure of avian liver is essentially the same as that of mammals, but lobulation is less distinct. Accumulations of lymphocytes around vessels and between liver cell cords are conspicuous. Lymphocytes often are intermingled with eosinophilic leukocytes, which by themselves may form foci within the liver parenchyma.

The sites of lysosomal acid hydrolase activity in liver tissue are the parenchymal cells, Kupffer cells, and bile duct epithelium, as is the case in mammals. Here also, lysosomes within parenchymal cells are located in pericanalicular regions.

(1) Parenchymal cells

(a) Acid phosphatase activity

Very significant B-glycerophosphate phosphatase activity is present in the numerous, small lysosomes of the Japanese quail and magpie. Somewhat less intense activities are present in lysosomes of horned owl, chicken and robin (Fig. 92).

Magpie possesses very high activity of Naphthol AS-TR-phosphate acid phosphatase, and chicken (Fig. 91) and horned owl display moderate activity for this isoenzyme in numerous small lysosomes. Activities, are low and moderate, respectively, in robin and Japanese quail (Fig. 96).

(b) Glucuronidase activity

Activity of glucuronidase is moderate in lysosomes of Japanese quail and magpie (Fig. 95), low in chicken and robin, and totally absent in horned owl.

(c) Glucosaminidase activity

Low enzyme activities are present in all birds and lysosomes are very small. The chicken liver is devoid of activity.

(d) Esterase activity

High a-Naphthyl acetate esterase activity is present in both granular and diffuse form in Japanese quail, magpie, chicken and robin (Fig. 97). In horned owl this activity is found in numerous medium-sized lysosomes.

Moderate Naphthol AS-acetate esterase activity, confined to numerous small granules, is found in chicken, horned owl, magpie, and Japanese quail, while only low activity is found in the robin.

(2) Kupffer cells

(a) Acid phosphatase activity

Activity of B-glycerophosphate phosphatase is very high in numerous small to large lysosomes in robin (Fig. 92), magpie and chicken. Japanese quail and horned owl, however, possess this activity in a few medium-sized lysosomes only.

All birds examined possess very high Naphthol AS-TR phosphate phosphatase activity (Figs. 91, 96). Lysosomes with this activity are numerous and medium-sized in Japanese quail (Fig. 96), and magpie, numerous and small in horned owl and robin, and few and small in the chicken.

(b) Glucuronidase activity

Lysosomes containing this enzyme activity are few and vary in size from small to medium. Activity is moderate to high in Japanese

quail, magpie (Fig. 95), and robin, and is low in chicken. The horned owl possesses no glucuronidase activity.

(c) Glucosaminidase activity

Moderate to high activity for this enzyme is noted in Japanese quail, magpie, horned owl, chicken, and robin. In all birds examined, lysosomes are few and small.

(d) Esterase activity

Granular and diffuse cytoplasmic activity for a-Naphthyl-acetate esterase is present in Kupffer cells. Activity is moderate to high in magpie and chicken, whereas, it is particularly low in robin (Fig. 97), horned owl, and Japanese quail.

Numerous small lysosomes, with moderate to high Naphthol AS-acetate esterase activity are present in Japanese quail, magpie, chicken, and horned owl. The robin, possesses very high activity for this enzyme, in a few small lysosomes.

C. Pancreas:

The avian pancreas is a tubuloacinous gland, but, unlike the mammalain pancreas, it is devoid of pronounced lobulation. Perivascular lymph nodules are not infrequent in the avian pancreas and islets of Langerhans are, particularly in chicken, more numerous than in mammals. Exocrine acinar cells, duct epithelial cells, and endocrine islet cells are, as in mammals, the sites of greatest lysosomal acid hydrolase activity.

(1) Islet cells

(a) Acid phosphatase activity

Low B-glycerophosphate phosphatase activity in magpie is restricted to numerous small lysosomes. In contrast, very diffuse activity is noted in islet cells in robin pancreas.

Moderate levels of Naphthol AS-TR-phosphate phosphatase activity, confined to small but numerous lysosomes, are present in Japanese quail and chicken, while lower levels of activity are found in magpie. Robin (Fig. 103), and horned owl show diffuse low activity of this isozyme.

(b) Glucuronidase activity

Low and diffuse activities are found in magpie and robin (Fig. 104) for this enzyme. Japanese quail, horned owl, and chicken on the other hand, possess no glucuronidase activity.

(c) Glucosaminidase activity

Activity of this enzyme is moderate in the robin, but low in the Japanese quail, horned owl, and chicken. Magpie possesses no glucosaminidase activity.

(d) Esterase activity

Generally, a-Naphthyl acetate esterase activity is low in islet cells. Reaction product is deposited as numerous granules in Japanese quail, and in diffuse form in the magpie and robin. Horned owl and chicken, noticeably, are negative for this enzyme activity.

Naphthol AS-acetate esterase activity is present as numerous granules of low activity in Japanese quail, chicken, and robin, and few granules of low activity in magpie and horned owl.

(2) Acinar cells

(a) Acid phosphatase activity

Japanese quail, chicken (Fig. 105), and robin possess moderate to high acid phosphatase activity in numerous small lysosomes, while magpie and horned owl possess few lysosomes with low activity.

(b) Glucuronidase and glucosaminidase activities

Significant glucuronidase activity in the birds studied is limited to the chicken, and the significant activity of glucosaminidase is reserved for Japanese quail. Both activities are low.

(c) Esterase activity

All birds examined (Japanese quail, magpie, horned owl, robin, chicken), possess low to moderate diffuse a-Naphthyl acetate esterase activity. Similarly, Naphthol AS-acetate esterase activity is low and of granular form in all birds; granules are numerous in Japanese quail and robin, and few in magpie, horned owl and chicken.

(3) Pancreatic duct epithelium

(a) Acid phosphatase activity

Small lysosomes possessing moderate to high enzyme activity are present in chicken. Low activity is found in Japanese quail, while magpie and robin, respectively, only possess low activities of B-glycerophosphate phosphatase and Naphthol-AS-TR-phosphate phosphatase isoenzymes. Horned owl in contrast, possesses no acid phosphatase activity.

(b) Glucuronidase activity

Chicken possesses moderate activity and robin possesses low activity for this enzyme, while the remaining birds possess no glucuronidase

activity.

(c) Glucosaminidase activity

Chicken is the only bird possessing any glucosaminidase activity in duct epithelium.

(d) Esterase activity

Diffuse cytoplasmic a-Naphthyl acetate esterase activity is of high intensity in chicken, and is of low intensity in magpie and robin. Japanese quail and horned owl are negative.

Low activity for Naphthol AS-acetate esterase in granular form is noted in chicken, robin, magpie and horned owl. Activity is absent, however, in Japanese quail.

D. Spleen:

The avian spleen is surrounded by a fibrous and muscular capsule from which sparse trabeculae penetrate into the splenic pulp. As in the mammalian spleen, red pulp consists of a framework of reticular cells and their processes and interstices filled with lymphocytes, monocytes, plasma cells, and macrophages, perfused by formed elements of the circulating blood. White pulp is characterized by germinal centres of lymphatic tissue, each surrounded by a cuff of small lymphocytes. An artery, the sheathed artery, is usually seen in these splenic lymphoid nodules. The reticulum framework found in the red pulp is continuous with that of the white pulp, where it is particularly dense about the sheathed arteries.

Investigation of lysosomal acid hydrolase activity in avian spleen revealed that the macrophages of red and white pulps, and the lympoid cells of white pulp are the sites of greatest activities.

(1) Red and White pulp macrophages

(a) Acid phosphatase activity

Magpie (Fig. 102), horned owl, and chicken (Fig. 98) possess numerous small to medium-sized lysosomes with moderate to high B-glycerophosphate acid phosphatase activity. Robin (Fig. 99), although very similar to the other birds with respect to Naphthol AS-TR-phosphate phosphatase activity, possesses only few lysosomes with high B-glycerophosphate phosphatase activity. The horned owl possesses Naphthol AS-TR phosphate phosphatase only in diffuse form.

(b) Glucuronidase activity

Robin red pulp macrophages are the only cells with numerous lysosomes and moderate glucuronidase activity. Lysosomes are few, and activity is only moderate in chicken and magpie red pulp macrophages, and magpie white pulp macrophages. Chicken and robin possess only low activities of glucuronidase in white pulp macrophages. Horned owl, in contrast, possesses no glucuronidase activity.

(c) Glucosaminidase activity

Glucosaminidase activity is moderate to high in small to medium-sized lysosomes in red pulp macrophages of horned owl (Fig. 101), chicken and robin. Lysosomes are few in the first two birds but numerous in the third. The magpie possesses low enzyme activity in a few small lysosomes.

In white pulp macrophages, on the other hand, lysosomes are few in all birds, and enzyme activity is low in magpie, horned owl, and robin, whereas it is moderate in the chicken.

(d) Esterase activity

Only low a-Naphthyl acetate esterase activity is present in red and white pulp macrophages of magpie, red pulp macrophages of chicken and robin (Fig. 100), and white pulp macrophages of horned owl. No other activities for this isoenzyme are present.

A few granules with low Naphthol AS-acetate esterase activity are present in magpie and robin. Horned owl and chicken, in contrast, are totally devoid of this activity.

(2) Red pulp cells (Cords of Billroth)

(a) Acid hydrolase activities

Most birds (magpie, horned owl, chicken (Fig. 98), robin) possess low acid phosphatase activity in these cells. Chicken, in addition,shows low activity of glucosaminidase. No other enzyme activities are noted.

(3) White pulp lymphoid cells

(a) Acid hydrolase activities

Moderate B-glycerophosphate phosphatase activity is present in chicken (Fig. 98); there is low activity in horned owl, and no activity is present in magpie (Fig. 102) and robin. Activity of Naphthol AS-TR-phosphate phosphatase is present at moderate to high intensity in chicken and robin, whereas, only low activity exists in magpie.

Glucuronidase activity is noticeably absent from all birds examined, and glucosaminidase activity is present only in robin, at a moderate level. Esterase activity is totally absent in all birds investigated, apart from low activity in the horned owl.

E. Duodenum:

The avian duodenum is very similar structurally to the mammalian duodenum. Some of the differences in appearance are the longer (1.5 mm.) villi which are covered by a simple columnar epithelium bearing a cuticular border, the presence in the epithelial lining of numerous goblet cells, and the presence within the stroma of the villi, in addition to the usual elements, of a central lacteal surrounded by bundles of smooth muscle. The lamina propria contains diffuse lymphatic tissue and often numerous eosinophils. Duodenal glands or Brunner's glands, are not present in avian duodenum.

Lysosomal acid hydrolase activity in duodenal villous epithelial cells characteristically decreases somewhat in activity from the apex to the base of the villus. Apical villous epithelial cells and lamina propria macrophages consistently are the sites of major lysosomal acid hydrolase activity.

(1) Apical villous epithelium

(a) acid phosphatase activity

Japanese quail, robin (Fig. 106), magpie, and horned owl possess numerous small lysosomes containing moderate to high B-glycerophosphate phosphatase activity. The chicken (Fig. 112) possesses few lysosomes, of moderate staining intensity for this enzyme. In contrast, the chicken (Fig. 113) and robin (Fig. 107) possess numerous lysosomes with moderate Naphthol AS-TR phosphate phosphatase activity, while only few lysosomes are present in the magpie. The horned owl shows low cytoplasmic activity of diffuse distribution for this latter isoenzyme.

(b) Glucuronidase activity

The Japanese quail displays numerous small lysosomes with high glucuronidase activity; magpie and robin (Fig. 108) possess a few small lysosomes with low glucuronidase activity; the chicken features diffuse low glucuronidase activity. The horned owl in contrast, has no glucuronidase activity in this site.

(c) Glucosaminidase activity

Principally, glucosaminidase activity is present in small numerous lysosomes at moderate to high intensity, in Japanese quail and chicken. Fewer lysosomes with lesser enzyme activity are present in horned owl and robin (Fig. 109). The magpie is without glucosaminidase activity.

(d) Esterase activity

High, diffuse cytoplasmic activity of a-Naphthyl acetate esterase and moderate to high granular activity of Naphthol AS-acetate

esterase are present in all the birds investigated (Figs. 110, 111, 114).

(2) Lamina propria macrophages

(a) Acid phosphatase activity

Japanese quail, chicken (Fig. 112), and horned owl possess numerous small lysosomes with moderate to high B-glycerophosphate phosphatase activity. Magpie and robin (Fig. 106), on the other hand, possess low and moderate activity for this isoenzyme, located in small numbers of lysosomes.

High activity in small, numerous lysosomes of Naphthol AS-TR phosphate phosphatase is noted in Japanese quail, magpie, chicken, and robin. The horned owl is negative for this isoenzyme activity.

(b) Glucuronidase activity

Lysosomes possessing this enzyme activity are small and few. Activity is high in magpie, moderate in Japanese quail and low in robin (Fig. 108) and chicken. Horned owl is negative for this activity.

(c) Glucosaminidase activity

Moderate to high enzyme activity is restricted to numerous small lysosomes in Japanese quail and to a few lysosomes in magpie, horned owl, chicken and robin (Fig. 109).

(d) Esterase activity

All birds examined possess low, granular activity for Naphthol-AS-acetate esterase (Figs. 111, 114). a-Naphthyl acetate esterase activity, also in granular form, is moderate and high in horned owl and magpie, respectively, and is low in chicken and robin (Fig. 110).

F. Brain:

The investigation of lysosomal acid hydrolases in avian brain was restricted, as in mammals, to the cerebral hemispheres and the cerebellum. The cells of principle interest in this investigation were cortical neurones of cerebral hemispheres, Purkinje cells of cerebellum, neuroglial cells, endothelial cells of blood capillaries, and perithelial microglia surrounding blood vessels.

(1) Cortical neurons

(a) Acid phosphatase activity

Japanese quail, chicken, and robin (Fig. 83) possess moderate B-glycerophosphate phosphatase activity confined to numerous small lysosomes. Similarly, Naphthol AS-TR-phosphate phosphatase activity is present in small, numerous lysosomes in chicken (Fig. 82) and Japanese quail. Cortical neurons of the robin only possess a few lysosomes with this activity.

(b) Glucuronidase activity

Of the birds examined, chicken alone possesses low diffuse glucuronidase activity.

(c) Glucosaminidase activity

Robin and chicken possess low activity of glucosaminidase in small lysosomes, as well as diffuse cytoplasmic staining. Japanese quail on the other hand, is dificient in this enzyme activity.

(d) Esterase activity

Activity of a-Naphthyl acetate esterase, is restricted to robin and chicken, and is of low to moderate intensity. Japanese quail

is devoid of this enzyme activity.

The robin is the only bird possessing Naphthol AS-acetate esterase activity in numerous granules (Fig. 84). Both chicken and Japanese quail are negative for this enzyme.

(2) Purkinje cells

(a) Acid phosphatase activity

Acid phosphatase activity of low intensity is present only in chicken and Japanese quail, confined to a few small lysosomes.

(b) Glucuronidase and glucosaminidase activities

Only chicken possesses these enzyme activities, and here they are of low intensity and of diffuse distribution.

(c) Esterase activity

Low granular and diffuse cytoplasmic activity with the a-Naphthyl acetate esterase method is found only in robin and chicken. Moderate activity for Naphthol AS-acetate esterase, is present only in the robin.

(3) Neuroglial cells

(a) Acid phosphatase activity

Generally, acid phosphatase activity is low, and lysosomes containing this activity are small and sparse in the chicken (Fig. 82), robin (Fig. 83), and Japanese quail. The robin, lacks Naphthol AS-TR-phosphate phosphatase activity.

(b) Glucuronidase and Glucosaminidase activities

Both of these enzyme activities are negative in all of the

birds investigated.

(c) Esterase activity

Low a-Naphthyl-acetate esterase activity of diffuse distribution is present in the chicken, whereas, Naphthyl AS-acetate esterase activity of low intensity, in granular form, is present in Japanese quail.

(4) Capillary endothelial cells

(a) Acid hydrolase activities

The only significant acid hydrolase activities of endothelial cells are low acid phosphatase activity in a few, small lysosomes in chicken, low B-glycerophosphate phosphatase activity in robin, and diffuse, low a-Naphthyl acetate esterase activity in Japanese quail. All other enzyme activities are absent.

(5) Perithelial microglia

(a) Acid phosphatase activity

Acid phosphatase activity, is moderate to high in robin (Fig. 82), and low in Japanese quail and chicken (Fig. 82). Lysosomes tend to be small.

(b) Glucuronidase activity

The Japanese quail possesses low activity for this enzyme in a few small lysosomes. The robin, also, possesses similarly low activity, but in a diffuse form.

(c) Glucosaminidase activity

Numerous small lysosomes with very high glucosaminidase activity are present in Japanese quail. In contrast, chicken and robin possess few small lysosomes with low activity for this enzyme.

(d) Esterase activity

a-Naphthyl acetate esterase activity of moderate intensity is present in granular form in the chicken. Esterase activity, on the other hand, is low and diffuse in the robin. In contrast, activities for Naphthol AS-acetate esterase, are low in robin (Fig. 84), and totally absent from chicken and Japanese quail.

III. REPTILES

Two species of reptiles, the turtle and the chameleon, were investigated for lysosomal acid hydrolase activities.

A. Kidney:

The reptilian kidney, like those of mammals and birds, is metanephric in type. Great variation of size and shape of this organ exist in reptiles. Turtle generally possesses a short, relatively compact kidney with internal lobulations. The nephron consists of the characteristic Malpighian corpuscle, Bowman's capsule, and tubular segments. The proximal convoluted tubule is in continuity with Bowman's capsule via a short "neck piece". At the distal portion of the proximal convoluted tubule a third segment provides a bridge to the distal convoluted tubule which then proceeds toward the collecting tubule.

A true portal circulation exists in the reptilian kidney. Portal vessels from the posterior extremities and posterior part of the body, furnish rich capillary ramifications around the kidney tubules, but none are provided to the glomerulus. Renal arterial blood provides a blood supply to this latter structure. The most significant lysosomal acid hydrolase activities of kidney are located in the proximal convoluted tubules and the collecting tubules in both the turtle and the chameleon.

(1) Proximal convoluted tubules

(a) Acid phosphatase activity

In the turtle B-glycerophosphate phosphatase activity of high intensity is displayed in numerous small to medium-size lysosomes. The corresponding isoenzyme activity in the chameleon is of moderate intensity and is present in a few small lysosomes. Both turtle (Fig. 119) and chameleon (Fig. 123) possess intense activity of Naphthol AS-TR phosphate phosphatase in numerous medium-sized lysosomes.

(b) Glucuronidase activity

Activity for this lysosomal enzyme is low in the turtle, and is totally absent in the chameleon.

(c) Glucosaminidase activity

The turtle (Fig. 120) possesses numerous small lysosomes with high glucosaminidase activity. In the chameleon, however, this activity is markedly reduced.

(d) Esterase activity

Granular deposits of reaction product of moderately intense activity of a-Naphthyl acetate esterase (Fig. 124) and Naphthol AS acetate esterase are shown in the chameleon. Turtle, however, possesses high activity only for the former isoenzyme (Fig. 121). Activity of the latter isoenzyme is low in this species.

(2) Collecting tubules

The turtle only, was examined for lysosomal acid hydrolase activities. Acid phosphatase, glucosaminidase, and esterase activities are low in the turtle. Glucuronidase activity, on the other hand, is absent from these tubules.

(3) Collecting ducts

Collecting ducts in kidney of turtle contain low activities for acid phosphatase, glucosaminidase, and esterase, while glucuronidase activity is absent.

B. Liver:

The liver of reptiles is a reticular or net-like glandular structure which, unlike the mammalian liver, possesses no lobulations. Instead, plates of liver cells, usually two cell layers thick, form a branching meshwork between which capillaries of the venous and arterial blood supply are located.Bile capillaries are located between liver cells at a distance one-half a cell thickness removed from the sinusoidal space. Very fine branches of the hepatic artery

are seen to communicate directly with sinusoidal spaces. Two further characteristics of reptilian liver are the presence often, of many granules within hepatic cells, especially in the proximity of bile capillaries, and the random distribution of pigment cells throughout the liver parenchyma.

Lysosomal acid hydrolase activity in reptilian liver is found principally in liver parechymal cells, Kupffer cells, and bile duct epithelium.

(1) Parenchymal cells

(a) Acid phosphatase activity

Both the turtle (Figs. 115,116) and the chameleon (Fig. 125) possess moderate acid phosphatase activity. Lysosomes, however, are few in the chameleon and numerous in the turtle. Difficulty was experienced in differentiating B-glycerophosphate phosphatase activity from granules normally present in parenchymal cells. The highly chromogenic reaction product formed in the Naphthol AS-TR phosphate phosphatase reaction, on the other hand, allows for the ready differentiation of acid phosphatase-containing lysosomes and pigment-containing granules in these cells.

(b) Glucuronidase activity

Only the turtle (Fig. 117) possesses low glucuronidase activity.

(c) Glucosaminidase activity

Lysosomes are numerous, small, and possess moderate glucosaminidase activity in the turtle (Fig. 118). The chameleon, in contrast, possesses no glucosaminidase activity.

(d) Esterase activity

The a-Naphthyl acetate esterase activity of these cells is of moderate intensity in both turtle and chameleon (Fig. 126). a-Naphthol AS acetate esterase activity, on the other hand, is of moderate intensity only in the chameleon, no activity being present in the turtle.

(2) Kupffer cells

(a) Acid phosphatase activity

Only the chameleon (Fig. 125) possesses B-glycerophosphate phosphatase activity, and this is of low intensity. Moderate and low to moderate activities, respectively, are present in the chameleon and the turtle (Fig. 116), however, for Naphthol AS-TR phosphate phosphatase. In the turtle this activity was in granular and diffuse form in the cytoplasm.

(b) Glucuronidase activity

High glucuronidase activity is present in numerous small lysosomes in the turtle (Fig. 117), while only low activity is present in the chameleon.

(c) Glucosaminidase activity

The turtle (Fig. 118) possesses moderate activity for this enzyme, whereas the chameleon is devoid of this enzyme activity.

(d) Esterase activity

Esterase activity is low in the chameleon (Fig. 126), and the turtle only possesses moderate a-Naphthyl acetate esterase activity.

(3) Bile duct epithelium

(a) Acid phosphatase activity

The B-glycerophosphate phosphatase activity is low in the chameleon and absent in the turtle. Naphthol AS-TR phosphate phosphatase, on the other hand, is moderate in the turtle and absent in the chameleon.

(b) Glucuronidase and glucosaminidase activities

Glucuronidase activity is negative in both reptilian species. Glucosaminidase activity, absent in the chameleon, is present at low to moderate intensity in the turtle.

(c) Esterase activity

Activity for a-Naphthyl acetate esterase is low to moderate in both the chameleon and the turtle. The chameleon only, however, possesses Naphthol AS acetate esterase activity

at low to moderate intensity.

C. Spleen:

The spleen of reptiles is primarily a lymphoid organ, and clearly defined lymphoid nodules are present in the spleens of both the turtle and the chameleon. Erythropoietic activity is reported to be present in the spleens of both reptiles. This activity is believed to be more prominent in the spleen of the turtle than the chameleon.

Lysosomal acid hydrolase activity is most intense in the macrophages of both red and white pulps.

(1) Macrophages of the red pulp

(a) Acid phosphatase activity

The turtle possesses few small lysosomes with moderate activities of B-glycerophosphate phosphatase and Naphthol AS-TR phosphate phosphatase (Fig. 127). In contrast, the chameleon possesses only low Naphthol AS-TR phosphate phosphatase activity in few small lysosomes.

(b) Glucuronidase activity

Glucuronidase activity is present, at low intensity, only in the turtle (Fig. 128).

(c) Glucosaminidase activity

Moderate and low glucosaminidase activities, respectively, are present in the turtle (Fig.129) and the chameleon. Lysosomes are small and sparse in both species.

(d) Esterase activity

Moderate to high diffuse a-Naphthyl acetate esterase activity is present in both the turtle and the chameleon. Activity of Naphthol AS acetate esterase, in contrast, is low in both species.

(2) Cords of Billroth

Lysosomal acid hydrolase activities, in cells resembling mammalian cords of Billroth, are not noted in the turtle or the chameleon.

(3) Macrophages of the white pulp

Lysosomal acid hydrolase activities in these cells are almost identical to the activities noted in the macrophages of the red pulp. The turtle in addition, however, possesses numerous small lysosomes with high glucosaminidase activity (Fig. 129).

(4) Lymphoid cells

Lysosomal acid hydrolase activities of lymphoid cells in the spleens of both turtle and the chameleon are very low or absent.

D. Pancreas:

The reptilian pancreas, like that of the mammals, is composed of exocrine alveolar glandular tissue, and associated ducts, and endocrine Islets of Langerhans. The Islets of Langerhans in the turtle and the chameleon, are more numerous than in the mammalian species.

(1) Islet cells

(a) Acid phosphatase activity

Lysosomal Naphthol AS-TR phosphate phosphatase activity is of moderate intensity in numerous small lysosomes. B-glycerophosphate phosphatase activity, on the other hand, is confined to few lysosomes and is of only low intensity in the turtle. The chameleon, characteristically, does not possess any acid phosphatase activity.

(b) Glucuronidase activity

Low activity of glucuronidase is present in the turtle, whereas, no such activity is present in the chameleon.

(c) Glucosaminidase activity

Glucosaminidase activity is moderate to high in the turtle (Fig. 135) and is low in the chameleon.

(d) Esterase activity

Turtle (Fig. 127) possesses only a-Naphthyl acetate esterase activity, whereas, chameleon possesses only Naphthol AS acetate esterase activity.

(2) Acinar cells

Acid phosphatase activity is low in both the turtle and the chameleon and glucuronidase activity is totally absent from both species. Chameleon only, possesses low glucosaminidase activity. Moderate and low diffuse activities of a-Naphthyl acetate esterase are found in the turtle and the chameleon respectively. Numerous small granules of moderate Naphthol AS acetate esterase activity, on the other hand, are found in both the chameleon and the turtle acinar cells.

(3) Pancreatic duct epithelium

The chameleon possesses low Naphthol AS acetate esterase activity, but no other lysosomal acid hydrolase activity. The turtle, on the other hand, possesses low activities for Naphthol AS-TR phosphate phosphatase, glucuronidase, glucosaminidase (Fig. 135), and a-Naphthyl acetate esterase (Fig. 122). Activities for B-glycerophosphate phosphatase and a-Naphthyl AS acetate esterase are absent, however.

E. Duodenum:

The reptilian intestine is lined by a single layer of tall columnar epithelial cells. The turtle possesses no distinct large or small intestine, instead an intestinal tract consisting of a tube of uniform diameter for the entirety of the gut is present. Histologically also, the appearance does not vary throughout its entire length. Villi are absent, but these are in part replaced by longitudinal folds of the mucosa which extends the full length of the intestine. Crypts of Lieberkühn, similar to those of mammals, are not seen in these reptilian species. The lamina propria is highly cellular and the cell type is predominantly lymphoreticular. The histochemical investigation of the anterior portion of the reptilian intestine is restricted to the epithelial cells lining the lumen of the intestine, and to the macrophages of the lamina propria.

(1) Epithelial cells

Moderate acid phosphatase (Fig. 132) and esterase (Fig. 131)

activities and very low glucosaminidase activity are present in lysosomes of the columnar epithelial cells. Glucuronidase activity is absent from these cells.

(2) Lamina propria macrophages

Lysosomes in macrophages of the lamina propria are small and few in number. Very high activity for Naphthol AS-TR phosphate phosphatase is present,however, in these lysosomes. In contrast, the activity of B-glycerophosphate phosphatase is very low. Glucuronidase and a-Naphthyl acetate esterase (Fig. 131) activities are low, while glucosaminidase and Naphthol AS acetate esterase activities are absent.

F. Brain:

The brain, both of the turtle and the chameleon, is, compared to the mammalian brain, primitive. Sulci and fissures of the cerebral hemispheres are rudimentary. Further, relative sizes of cerebral hemispheres and cerebellum, as compared to the whole brain, are smaller than is the case in mammals.

The study of the distribution of lysosomal acid hydrolase activities, in both the turtle and the chameleon, was limited to cell types very similar to those investigated in the brains of mammals and birds.

(1) Cortical neurons

The chameleon is devoid of any lysosomal acid hydrolase activity in these cells. The turtle, on the other hand, possesses moderate to high acid phosphatase (Fig. 136) and glucosaminidase (Fig. 137)activities in numerous small lysosomes. Glucuronidase activity is low, whereas,esterase activity is entirely absent.

(2) Purkinje cells

No lysosomal acid hydrolase activity is present in these cells.

(3) Glial cells

Moderate Naphthol AS-TR phosphate phosphatase activity, localized to numerous small lysosomes, and low to moderate glucosaminidase activity in a few lysosomes, are seen in the glial cells.

Other lysosomal acid hydrolases are, however, absent.

(4) Endothelial cells

The endothelial cells of the turtle brain are devoid of all lysosomal acid hydrolase activities.

(5) Perithelial microglia

While a-Naphthyl acetate esterase,Naphthol AS-TR phosphate phosphatase, and glucuronidase activities are relatively low in perithelial microglia of the turtle, glucosaminidase activity is quite high in numerous small lysosomes in these cells.

IV AMPHIBIA

Lysosomal acid hydrolase activities of various organs of species of amphibia

Lysosomal acid hydrolase activities were studied in the liver, kidney, spleen, duodenum, pancreas, and brain of grass frog, tree frog, salamander, and newt.

A. Kidney:

The amphibian kidney is of mesonephric type, and is made up of structural units consisting of a glomerular tuft of capillaries, surrounded by Bowman's capsule, a short neck piece leading to the proximal convoluted tubule, an intermediate tubular segment which continues to the distal convoluted tubule, a collecting tubule, and a Wolffian duct or ureter. In male amphibia, the anterior portion of the kidney is in close apposition to the testis and functions in the transport of spermatozoa. As in reptiles and birds, the amphibian kidney is endowed with a dual renal circulation - a renal arterial blood supply to the glomeruli and a venous blood supply to the renal tubules from the renal portal vein.

(1) Proximal tubules

Lysosomal acid phosphatase activities of grass frog and tree frog (Fig. 138) are very high, concentrated in numerous small to medium sized lysosomes. Moderate to high glucuronidase (Figs. 139, 142) and glucosaminidase (Fig. 140) activities are also present in proximal tubule cells of these species. Esterase activity is very high in both amphibia (Figs. 141, 143) as indicated by a diffuse deposition in cytoplasm of a granular reaction product.

B. Liver:

The amphibian liver grossly and microscopically varies little from the reptilian liver described previously.

(1) Parenchymal cells of liver

(a) Acid phosphatase activity

The grass (Fig. 144) and tree frogs possess moderate to high acid phosphatase activity in numerous small lysosomes. The salamander, on the other hand, possesses only moderate Naphthol-AS-TR-phosphate phosphatase activity in small to medium sized lysosomes (Fig. 150).

(b) Glucuronidase activity

Moderate glucuronidase activity is present in small lysosomes in the grass frog (Fig. 145), tree frog (Fig. 146), the newt (Fig. 159), and the salamander (Fig. 160). Lysosomes are numerous only in the grass frog and the newt, however.

(c) Glucosaminidase activity

Activity of glucosaminidase is high in the salamander (Fig. 147) and low in the grass frog. The small lysosomes, however, are sparse in the former, and numerous in the latter species of amphibia. The tree frog is devoid of this enzyme activity.

(d) Esterase activity

Esterase activity is moderate to high in the grass and tree frogs (Fig. 151), but is low in the salamander.

(2) Kupffer cells

(a) Acid phosphatase activity

Lysosomes are few but contain very high B-glycerophosphate phosphatase activity in both grass (Fig. 144) and tree frogs (Fig. 149). Naphthol AS-TR-phosphate phosphatase activity is also high in the grass and tree frogs, as well as in the salamander (Fig. 150). Lysosomes are numerous in the tree frog and the salamander, but are sparse in the grass frog.

(b) Glucuronidase activity

Numerous small lysosomes with moderate to high glucuronidase activity are present in both grass (Fig. 145) and tree frogs (Fig. 146). In the salamander (Fig. 148) and newt (Fig. 159), activity of this enzyme is also moderate to high, but lysosomes are small and sparse.

(c) Glucosaminidase activity

Glucosaminidase activity is moderate in the grass and tree frogs, and high in the salamander (Fig. 147). Lysosomes are small to medium in size in the Kupffer cells of these species.

(d) Esterase activity

Esterase activity of high intensity is present in the grass and tree frogs (Fig. 151). The salamander, in contrast, shows only a-Naphthyl acetate esterase activity, at moderate levels, in diffuse and granular form.

(3) Bile duct epithelium

(a) Acid phosphatase activity

Lysosomes of bile duct epithelial cells are small but numerous. These contain moderate to high acid phosphatase activity in the tree frog,

but only Naphthol AS-TR-phosphate phosphatase activity in the grass frog.

(b) Glucuronidase activity

Only the grass frog possesses this enzyme activity in the bile duct epithelium.

(c) Glucosaminidase activity

The grass frog possesses numerous small lysosomes with moderate glucosaminidase activity. The newt and tree frog, on the other hand, are devoid of this enzyme activity.

(d) Esterase activity

A granular reaction product of moderate to high density at sites of esterase activity, is present in the grass and tree frogs. The salamander is negative for this enzyme activity.

C. Spleen:

The amphibian spleen is predominantly a lymphoid organ, and shows great similarity in structure, grossly and microscopically, to the reptilian spleen. Similarly, the cellular elements with lysosomal acid hydrolase activities were the cells of the red and white pulps - the macrophages, cords of Billroth, and the lymphoid cells.

(1) Red pulp macrophages

(a) Acid phosphatase activity

Acid phosphatase activity is moderate to high in grass and tree frogs, salamander (Fig. 161), and newt (Fig. 162). The lysosomes are few in tree frog,salamander and newt, and numerous in grass frog.

They are small in all of the species studied.

(b) Glucuronidase activity

Grass frog possesses moderate activity for this enzyme localized to numerous, small lysosomes.

Newt and salamander (Fig. 160), on the other hand, possess moderate, diffuse activity in cytoplasm.

(c) Glucosaminidase activity

The salamander possesses numerous, very small lysosomes with high glucosaminidase activity. In contrast, the grass frog and the newt possess very low activity for this enzyme.

(d) Esterase activity

Grass frog and salamander, respectively, possess moderate and very low activities for esterase. The newt displays no esterase activity.

(2) Cords of Billroth

The cords of Billroth contain no significant lysosomal acid hydrolase activity in the species examined.

(3) White pulp macrophages

Lysosomes of macrophages of the white pulp show the same spectrum of acid hydrolase activity as occurs in macrophages of red pulp.

(4) Lymphoid cells

Lymphoid cells show very little lysosomal acid hydrolase activity in the amphibia studied.

D. Pancreas:

The amphibian pancreas is present in two portions, the dorsal

portion lying in the dorsal mesentery and the ventral portion lying between the small intestine and the liver. Microscopically, the substance of the pancreas consists of acini and associated excretory ducts, Islets of Langerhans, and numerous capillaries. Amphibian Islets cells, acinar cells, and duct epithelium were examined for content and distribution of lysosomal acid hydrolases.

(1) Islet cells

(a) Acid phosphatase activity

Acid phosphatase activity is moderate in the tree frog and low in the salamander. The grass frog possesses moderate Naphthol-AS-TR-phopshate phosphatase and low B-glycerophosphate phosphatase activities. Lysosomes, generally, are numerous and small in these cells.

(b) Glucuronidase activity

All amphibians studied are deficient in glucuronidase activity in this location.

(c) Glucosaminidase activity

Glucosaminidase activity is low in the tree frog and the salamander, and is negative in the grass frog.

(d) Esterase activity

The grass frog possesses moderate esterase activity. Esterase is very low or absent in the other amphibians.

(2) Acinar cells

(a) Acid phosphatase activity

Small lysosomes with low acid phosphatase activity are present

in grass frog, tree frog, and salamander.

(b) Glucuronidase activity

All amphibians studied are devoid of this enzyme activity.

(c) Glucosaminidase activity

Moderate activity, precisely localized to a few small lysosomes, is present in the salamander only.

(d) Esterase activity

The grass and tree frogs (Fig. 157) possess moderate esterase activity. Salamander, on the other hand, displays low, diffuse a-Naphthyl acetate esterase, and moderate Naphthol AS-acetate esterase activities, at this site.

(3) Pancreatic duct epithelium

(a) Acid phosphatase activity

The grass frog, tree frog, and salamander, possess small lysosomes with low acid phosphatase activity.

(b) Glucuronidase activity

Numerous small lysosomes, containing very high glucuronidase activity are displayed in the grass frog. All other amphibians studied are devoid of this enzyme activity.

(c) Glucosaminidase activity

High activity for this enzyme is present in the grass frog only. All other amphibians studied do not possess this enzyme activity.

(d) Esterase activity

a-Naphthyl acetate esterase activity is of low intensity and distributed throughout the cytoplasm in all the amphibians studied. Naphthol-AS-acetate esterase activity is present in granular form in all the amphibian species examined. This latter isozyme activity is low in tree frog and salamander, but is of high intensity in the grass frog.

E. Duodenum:

The intestinal epithelium of amphibia, characteristically, lacks true villi. Scattered throughout the intestinal mucosa and between lining epithelial cells, are numerous large and small lymphocytes, polymorphonuclear leukocytes, mast cells, and pigment cells. Crypts of Lieberkuhn make their first appearance in amphibia. These are noted in the salamander, but are absent in the frog. The lamina propria is composed of a lympho-reticular tissue. Macrophages are very abundant throughout this tissue.

Lysosomal acid hydrolase activities were seen in epithelial cells and macrophages of the lamina propria.

(1) Duodenal epithelium

(a) Acid phosphatase activity

All amphibian species studied display numerous small lysosomes with moderate to high Naphthol-AS-TR-phosphate phosphatase activity (Figs. 152, 155, 156). The tree frog and the newt possess high B-glycerophosphate phosphatase activity, while the grass frog and the salamander only display low activity for this isozyme.

(b) Glucuronidase activity

The newt, the tree frog (Fig. 154), and the grass frog display low glucuronidase activity in small lysosomes. Salamander, on the other hand, is devoid of this enzyme activity.

(c) Glucosaminidase activity

Lysosomes in all amphibians studied are numerous but small; glucosaminidase activity is moderate to high (Figs. 153, 163, 164).

(d) Esterase activity

Esterase activity of moderate to high intensity, is present in the grass and tree frogs, the salamander, and the newt.

(2) Macrophages of the lamina propria

(a) Acid phosphatase activity

The activity of B-glycerophosphate phosphatase is moderate in the grass frog, the tree frog, and the newt. The salamander is devoid of this isoenzyme activity. Naphthol-AS-TR-phosphate phosphatase activity is moderate to high in all the amphibians studied (Figs. 152, 155, 156).

(b) Glucuronidase activity

Moderate glucuronidase activity is present in the grass and tree frogs (Fig. 154), and the newt. Lysosomes are small in all three species; these are numerous in the frogs, but sparse in the newt. The salamander is devoid of glucuronidase activity.

(c) Glucosaminidase activity

The salamander (Fig. 164) displays very high activity for

glucosaminidase in numerous small lysosomes. The newt (Fig. 163) and the tree frog possess similarly high activity for this enzyme, but only in a few small lysosomes. Unlike the foregoing species, the grass frog (Fig. 153) shows moderate enzyme activity of diffuse cytoplasmic distribution.

(d) Esterase activity

Activities of a-Naphthyl acetate esterase and Naphthol-AS-acetate esterase are high in the tree frog and low in the salamander and grass frog. The newt is devoid of these esterase activities.

F. Brain:

The amphibian brain resembles that of reptiles and birds. Cerebral hemispheres and the cerebellum lack the corrugations, fissures, and sulci, seen in mammalian brain. For the purpose of this study, cells corresponding to those examined in mammalian brain, were studied for lysosomal acid hydrolase activity and distribution. These cells were cortical neurones, Purkinje cells, glial cells, endothelial cells of blood vessels, and perithelial microglia.

(1) Cortical Neurones

(a) Acid phosphatase activity

Numerous small lysosomes staining intensely for B-glycerophosphate phosphatase and Naphthol AS-TR-phosphate phosphatase are present in cortical neurones of the grass frog and the salamander (Fig. 166). These enzyme activities are low and lysosomes are sparse in the tree frog, while the newt is devoid of acid phosphatase activity.

(b) Glucuronidase activity

Lysosomal glucuronidase activity is low in all the amphibians studied.

(c) Glucosaminidase activity

Lysosomal glucosaminidase activity is present in the newt (Fig. 165) and the salamander. In these species, it is present at high intensity in numerous small lysosomes.

(d) Esterase activity

Moderate a-Naphthyl acetate esterase activity is present only in the tree frog. Naphthol-AS-acetate esterase activity, on the other hand, is moderate in the salamander, low in the grass frog, and low in the newt.

(2) Purkinje cells

Very low Naphthol AS-TR-phosphate phosphatase activity in the grass and tree frogs, and moderate Naphthol-AS-acetate esterase activity in the tree frog are the salient lysosomal acid hydrolase activities in the amphibia studied.

(3) Glial cells

Moderate B-glycerophosphate phosphatase activity in the grass frog, low Naphthol-AS-TR-phosphate phosphatase activity in the grass and tree frogs, and low Naphthol AS-acetate esterase activity in the tree frog, are the principal lysosomal acid hydrolase activities of amphibian glial cells.

(4) Endothelial cells of blood vessels

Lysosomal acid hydrolase activities are low in these cells in all the amphibians studied.

(5) Perithelial microglia

The dominant lysosomal acid hydrolase activities of perithelial microglia in the amphibians studied are: - moderate B-glycerophosphate phosphatase activity in numerous small lysosomes in the grass frog; low Naphthol AS-TR-phosphate phosphatase activity in a few small lysosomes in the grass and tree frogs; low a-Naphthyl acetate esterase activity in the tree frog; and low Naphthol-AS-acetate esterase activity in the grass and tree frogs.

V. FISH

The lysosomal acid hydrolase activities were studied in two species of fish:- Esox lucius and Stizostedion vitreum.

A. Kidney:

The kidney of fish is of the mesonephric type. Glomerular development, in the fresh water fish studied, is well advanced, as is the development of tubules and ducts. Reticular connective tissue, infiltrated by numerous lymphocytes, is present between the tubular elements of these kidneys. Lymphoid tissue becomes much more abundant in the cranial portions of these kidneys. Attempts were made, in deliniating lysosomal acid hydrolase activity, to differentiate the portions of the renal tubules into the proximal and distal convoluted tubules, the collecting tubules, and the collecting ducts.

(1) Proximal tubules

Both Exos lucius and Stizostedion vitreum possess numerous small to medium sized lysosomes with moderate to high acid phosphatase (Figs. 167, 168), and glucuronidase (Figs. 169, 170) activities. Glucosaminidase activity is high in Esox lucius and low in Stizostedion vitreum. Both fish possess moderate to high esterase activity in diffuse form (Figs. 171, 172).

(2) Collecting tubule

Sparse, small lysosomes with low acid phosphatase (Figs. 167, 168) and esterase (Figs. 171, 172) activities are present in both fish. Glucuronidase and glucosaminidase activities are not seen in these fish cells.

(3) Collecting ducts

While both Esox lucius and Stizostedion vitreum display moderate Naphthol AS-TR-phosphate phosphatase activity, the B-glycerophosphate phosphatase activity, at low levels, is present only in Esox lucius. Glucuronidase and glucosaminidase activities are negative in both fish. Low, diffuse a-Naphthyl acetate esterase activity is present only in Esox lucius, whereas, both fish display low Naphthol-AS-acetate esterase activity.

(4) Lymphoid tissue

Stizostedion vitreum shows extensive deposits of lymphoid tissue between the tubules of the kidney. High activities of acid phosphatase (Fig. 168), glucuronidase (Fig. 170), glucosaminidase, and esterase (Fig. 172), precisely localized, are present in these cells. Black pigment- and acid hydrolase-containing cell aggregates are present in the kidney of Stizostedion vitreum, but not in Esox lucius.

B. Liver:

The liver of fish lacks the lobulations found in mammalian liver. It is, instead, made up of laminae or plates of liver cells, which branch and anastamose with each other. Sinusoidal spaces, lined by numerous phagocytic Kupffer cells, occupy the intervening spaces between the liver cells. Liver cells of fish are large, poor in structural content, but rich in fat.

Lysosomal acid hydrolase activity in fish liver was most abundant in liver parenchymal cells, Kupffer cells, and bile duct epithelial cells.

(1) Parenchymal cells

(a) Acid phosphatase activity

Numerous lysosomes of variable size but of high B-glycerophosphate phosphatase activity are present in Esox lucius. Stizostedion vitreum, on the other hand, displays only sparse numbers of small lysosomes with moderate activity for this enzyme. Both species possess numerous lysosomes of medium size with moderate to high Naphthol AS-TR-phosphate phosphatase activity (Figs. 173, 174).

(b) Glucuronidase activity

The activity for this enzyme is moderate to high in both species of fish in numerous small to medium sized lysosomes (Fig. 175).

(c) Glucosaminidase activity

Glucosaminidase activity is moderate in Esox lucius but is low in Stizostedion vitreum.

(d) Esterase activity

Moderate to high esterase activity is present in both Esox lucius and Stizostedion vitreum liver parenchymal cells (Fig. 176).

(2) Kupffer cells

Lysosomes of Kupffer cells, generally, are few, but large, in Esox lucius and of medium size in Stizostedion vitreum.

Acid phosphatase (Figs. 173, 174), glucuronidase (Fig. 175), glucosaminidase, and esterase (Fig. 176) activities of moderate to high intensities are present in both species.

(3) Bile duct epithelium

(a) Acid phosphatase activity

Esox lucius possesses low activity of B-glycerophosphate phosphatase in contrast to Stizostedion vitreum which is devoid of this activity. Naphthol AS-TR-phosphate phosphatase activity is moderate in Esox lucius and low in Stizostedion vitreum.

(b) Glucuronidase activity

Low glucuronidase activity, confined to a few small lysosomes, is present in Esox lucius but not in Stizostedion vitreum.

(c) Glucosaminidase activity

Glucosaminidase activity is absent from bile duct epithelium in both species of fish.

(d) Esterase activity

Both Esox lucius and Stizostedion vitreum are devoid of a-Naphthyl acetate esterase activity. Naphthol AS-acetate esterase activity of low intensity is present, however, in both species.

C. Spleen:

A distinct splenic organ is present in both Esox lucius and Stizostedion vitreum. Lymphoid and erythroid cellular elements are present in this organ, however, the organization into white pulp and red pulps, as seen in the mammalian spleen, is not present in these organs. Stizostedion vitreum possesses numerous large pigment-containing cells in the splenic stroma, which are not present in Esox lucius. These cells were found to possess lysosomal acid hydrolase activities

as well as their complement of black pigment. The pigment-containing cells were not arranged in any systematic fashion in the spleen.

Lysosomal acid hydrolase activities are present in macrophages distributed throughout the spleen, in lymphoid cells also evenly distributed throughout the spleens of both species of fish, and in erythropoietic cells in these organs.

(1) Macrophages

Numerous, small to large, lysosomes are present in the macrophages of the spleen of Stizostedion vitreum. Contained within these lysosomes are moderate to high acid phosphatase (Fig. 181), glucuronidase, glucosaminidase (Fig. 182), and esterase activities. Esox lucius possesses only small to medium size lysosomes with high Naphthol AS-TR-phosphate phosphatase (Fig. 179) and Naphthol AS-acetate esterase activities, low to moderate B-glycerophosphate phosphatase, glucuronidase, glucosaminidase (Fig. 180) and esterase activities.

(2) Erythropoietic cells

Acid phosphatase activity of low intensity is present in few small lysosomes in both species of fish. Naphthol AS-acetate esterase activity, also is of low intensity and localized to a few small lysosomes in these cells. Low glucosaminidase activity is present only in Stizostedion vitreum, whereas, glucuronidase activity is absent in erythropoietic cells of this fish as well as Esox lucius.

(3) Lymphoid cells

Esox lucius and Stizostedion vitreum possess low to moderate Naphthol AS-TR-phosphate phosphatase activity. No other lysosomal acid

hydrolase activities are seen in these cells.

D. Pancreas:

Esox lucius and Stizostedion vitreum possess a compact pancreas, as opposed to the diffuse or disseminated pancreas, found in some other fish. The exocrine pancreas of Esox lucius, in addition to the acini and ducts, also displays masses of lymphocytes dispersed between the glandular elements. The endocrine Islets of Langerhans in both fish appear to be larger, but fewer in number, than are those of the mammalian pancreas. Esox lucius Islets of Langerhans also appear as extra pancreatic islets, forming the so-called "principal islands".

Lysosomal acid hydrolase activities were studied in the Islet cells, the acinar cells, and the pancreatic duct epithelium.

(1) Islet cells

B-glyceropphosphate phosphatase activity of moderate to high intensity is present in Esox lucius, whereas, only low and diffuse activity is seen in Stizostedion vitreum. Glucosaminidase activity is of moderate intensity in numerous small lysosomes in both fish. Glucuronidase activity, on the other hand, is absent in both species. Esox lucius possesses moderate to high a-Naphthyl acetate and Naphthol AS-acetate esterase activities, while Stizostedion vitreum only displays moderate Naphthol AS-acetate esterase activity.

(2) Acinar cells

Acid phosphatase activity is low in both Esox lucius and Stizostedion vitreum. The lysosomes, however, are numerous in the former species and few in the latter species. Glucuronidase

and glucosaminidase activities are absent in both fish. Stizostedion vitreum possesses moderate a-Naphthyl acetate esterase and Naphthol-AS acetate esterase activities, whereas, Esox lucius possesses moderate Naphthol AS acetate esterase activity, but only low a-Naphthyl acetate esterase activity.

(3) Pancreatic duct epithelium

(a) Acid phosphatase activity

Lysosomal B-glycerophosphate phosphatase activity is moderate in Stizostedion vitreum and low in Esox lucius. Lysosomes in both species are quite small. Naphthol AS-TR-phosphate phosphatase activity is moderate in both fish, however, lysosomes are sparse in Stizostedion vitreum and numerous in Esox lucius.'

(b) Glucuronidase activity

Both fish are devoid of this enzymic activity.

(c) Glucosaminidase activity

Only Stizostedion vitreum displays a few small lysosomes with low glucosaminidase activity.

(d) Esterase activity

Stizostedion vitreum possesses low a-Naphthyl acetate esterase activity in a few small lysosomes as well as some diffuse cytoplasmic deposits of reaction products. Esox lucius, in contrast, is devoid of this enzyme activity. Naphthol-AS-acetate esterase activity is present only in Stizostedion vitreum. The enzyme activity is moderate and is distributed as a granular reaction product throughout the cytoplasm.

E. Duodenum:

In general, the anterior portion of the intestine of Esox lucius and Stizostedion vitreum is a very simple structure compared to this portion of intestine in mammals. Villi are present in both species and branching is noted in some sections. Lymphoreticular tissue is present in the lamina propria of both species, but is more abundant in Stizostedion vitreum. Goblet cells are frequent in the epithelium of the gut.

The lining epithelium and the macrophages of the lamina propria possess most of the lysosomal acid hydrolase activities.

(1) Epithelial cells

High activity of B-glycerophosphate phosphatase is present in numerous small lysosomes in Esox lucius. Sparse lysosomes with low activity for this enzyme are present in Stizostedion vitreum. Numerous small lysosomes containing Naphthol AS-TR-phosphate phosphatase activity are present in both species of fish. Activity of this enzyme is high in Esox lucius (Fig. 177), and moderate in Stizostedion vitreum. Glucuronidase activity is low in Stizostedion vitreum localized to a few small lysosomes. This activity, however, is absent in Esox lucius. Glucosaminidase activity is of low intensity in Stizostedion vitreum and moderate intensity in Esox lucius (Fig. 178). In both fish this activity is present in numerous small to medium sized lysosomes. Esterase activity is moderate to high in both species of fish.

(2) Lamina propria macrophages

Lysosomes, with moderate to high acid phosphatase activity, are numerous and small to medium is size in both Esox lucius (Fig. 177)

and Stizostedion vitreum. Glucuronidase activity of moderate intensity is present in Esox lucius. This activity is not found in Stizostedion vitreum. Glucosaminidase activity of lamina propria macrophages is of very high intensity in Esox lucius (Fig. 178), but very low in Stizostedion vitreum. In both fish lysosomes were sparse and of small to medium size. Stizostedion vitreum possesses very high activity for a-Naphthyl acetate esterase while Esox lucius possesses only low activity for this isozyme. Naphthol AS-acetate esterase activity, moderate to high in intensity, is displayed by both species of fish.

F. Brain:

The brain of both Esox lucius and Stizostedion vitreum is small and primitive. The cerebral hemispheres and the cerebellum in both fish, in particular, are very simple and small structures. Cell types, similar in appearance to cortical neurones, Purkinje cells, glial cells, endothelial cells, and perithelial microglia of mammals, were studied for acid hydrolase content and distribution.

(1) Cortical neurons

Numerous small lysosomes with high B-glycerophosphate phosphatase activity are seen only in Esox lucius. Stizostedion vitreum displays none of this activity. Moderate Naphthol AS-TR-phosphate phosphatase activity is present in both species of fish in numerous small lysosomes. Glucuronidase, glucosaminidase, and esterase activities are very low or absent from both Esox lucius and Stizostedion vitreum.

(2) Purkinje cells

Only low, diffuse Naphthol AS-TR phosphate phosphatase activity

is displayed by both species of fish. Other lysosomal acid hydrolase activities are absent from these cells.

(3) Glial cells

Stizostedion vitreum possesses no lysosomal acid hydrolase activities. Esox lucius, on the other hand, possesses numerous small lysosomes with high Naphthol-AS-TR phosphate phosphatase activity. Moderate and low Naphthol AS acetate esterase and a-Naphthyl acetate esterase activities, respectively, are displayed by Esox lucius. The remaining lysosomal acid hydrolase activities are negative in Esox lucius.

(4) Endothelial cells

Esox lucius shows low acid phosphatase and low Naphthol AS-acetate esterase activities only. Stizostedion vitreum is negative for these as well as the remaining lysosomal acid hydrolase activities.

(5) Perithelial microglia

Esox lucius and Stizostedion vitreum possess moderate to high acid phosphatase activity. Lysosomes, however, were sparse in Stizostedion vitreum and numerous in Esox lucius. Stizostedion vitreum is devoid of glucuronidase and glucosaminidase activities, both activities of which are high in Esox lucius confined to numerous small to medium-sized lysosomes. Both fish are devoid of a-Naphthyl acetate esterase activity. Esox lucius, however, possesses moderate Naphthol AS-acetate esterase activity.

RESULTS

Modulations of Lysosomal Enzymes by Experimental Means

I. Induction:

A. Hormone Induction of a Lysosomal Enzyme

(1) Effect of testosterone proprionate on glucuronidase in mouse kidney and liver.

Kidney:

Subcutaneous injections of testosterone propionate (1.0 mg. dissolved in 0.1 ml. olive oil x 7d, q. 2d.) produced a marked increase in renal B-glucuronidase activity in adult male mice of Ajax strain (Figs. 223, 225, 227). In cells lining proximal tubules and in intercalated cells of collecting tubules, there was a manyfold increase in both lysosomal and cytoplasmic B-glucuronidase activity. Control mice (Fig. 221), receiving olive oil injections only, showed a decrease in lysosomal B-glucuronidase activity in both locations, when compared to untreated controls.

Liver:

Administration of testosterone propionate failed to stimulate glucuronidase activity in mouse liver (Figs. 224, 226, 228). Whereas in untreated control mice (Fig. 220), lysosomes of liver cells and Kupffer cells possessed high B-glucuronidase activity, olive oil-treated control mice (Fig. 222) and testosterone-treated mice (Figs. 224, 226, 228), showed a considerable decrease in numbers of lysosomes and depression of B-glucuronidase activity.

(2) Effect of Oestrone on glucuronidase in mouse liver.

Subcutaenous administration of 0.05 mg. Oestrone dissolved in 0.25 ml. of olive oil produced no significant changes in lysosomal hydrolases in kidney and liver cells in mice (Figs. 229, 230, 231, 232). Olive oil-treated control mice (Fig. 221), compared to untreated control mice (Fig. 219), showed a depression of B-glucuronidase activity in lysosomes of proximal tubule cells and intercalated cells of collecting tubules. This effect was not seen in liver cell lysosomes.

B. Through Formation of New Lysosomes

(1) By agents that induce pinocytosis (i.e., dextran).

The intra-peritoneal administration, on nine successive days, of 50 mg. dextran (M.W., 160,000) in 0.9% saline, to mature male Ajax mice produces no significant changes in lysosomal acid hydrolases in cells of kidney (Fig. 245, 247, 249, 251), liver (Figs. 246, 248, 250, 252), pancreas, spleen, duodenum, or brain.

(2) By production of cytolysosomes by the administration of glucagon.

Treatment of 200 g. male Wistar rats with 50 u g. of glucagon per 100 g. body weight produces no significant changes in lysosomal acid phosphatase, B-glucuronidase, glucosaminidase, and esterase activities in kidney (Figs. 211, 213), or liver (Figs. 212, 214). There is no apparent increase in size of lysosomes in these tissues.

II. Inhibition:

Inhibition of a lysosomal enzyme (Acid phosphatase) in liver and kidney by the administration of N-2-fluorenylacetamide (AAF).

Lysosomal acid phosphatase activity is reduced approximately 50% in rat kidney (Figs. 235, 236) and duodenum following administration of a single oral dose of AAF. Lysosomal acid phosphatase activity is reduced to a much lesser degree in liver parenchymal cells (Figs. 239, 240), pancreatic islet and acinar cells, macrophages of spleen, and cortical neurons and Purkinje cells of brain.

B-glucuronidase activity in AAF-treated animals is unchanged in liver and brain cells. A slight decrease in activity is noted, however, in lysosomes of proximal tubule cells and in intercalated cells of kidney, pancreatic islet cells, splenic macrophages, and villous epithelium and macrophages of duodenum.

Glucosaminidase activity in AAF-treated animal appears to be slightly elevated in proximal and distal tubule cells, intercalated cells of collecting tubules and collecting duct cells of kidney. Lysosomal glucosaminidase activity of hepatocytes and Kupffer cells, splenic macrophages, duodenal epithelial cells, cortical neurons, Purkinje cells, and perithelial microglia of brain, also appears to be slightly elevated. Alpha-Naphthyl acetate esterase activity in AAF-treated rats is depressed slightly in proximal tubule cells, collecting tubule intercalated cells, and collecting duct cells of kidney. Liver cells and acinar cells of pancreas of AAF-treated rats show a similar slight depression in enzyme activity. The most noticeable depression of alpha-Naphthyl acetate esterase activity in AAF-treated rats is seen in splenic cords of Billroth and white pulp macrophages.

III. Effects of Drugs on Histochemically Demonstrable Lysosomal Hydrolases:

A. Cortisone

Administration of 10 mg. cortisone subcutaneously q. d., for ten days to adult male Wistar rats does not produce any significant changes in number, size, or intensity of histochemical staining for acid phosphatase, B-glucuronidase, glucosaminidase, and esterase activities in kidney (Figs. 203, 204, 205, 206) and liver (Figs. 197, 198, 199, 200).

B. Phenacetin

The consumption by rats of a diet containing 1.8% phenacetin over a period of 9 weeks did not produce significant quantitative changes in the size or number of lysosomes or in lysosomal enzymes in kidney (Figs. 185, 186, 187, 188), or liver (Figs. 191, 192, 193, 194).

IV. Effect of Phosphorus-Induced Hepatocellular Necrosis on Histochemically Demonstrable Lysosomal Acid Hydrolases:

Intragastric administration of a suspension of yellow phosphorus in mineral oil to 200 g. male Wistar rats produces mild peripheral zonal necrosis in liver, and some alterations in lysosomal hydrolase activities in kidney (Figs. 215, 217) and liver (Figs. 216, 218). There is no apparent activation of lysosomal acid hydrolases in areas of hepatocellular necrosis, although some diffusion of enzyme activity is noted.

An interesting additional finding in liver is the complete inhibition by phosphorus of acid phosphatase activity in Kupffer cells and in bile duct epithelium. B-glucuronidase activity is markedly reduced in renal proximal tubule cells, intercalated cells of collecting tubules, and cells of collecting ducts. Glucosaminidase activity, which is moderately intense in controls, is totally absent from lysosomes in in intercalated cells of collecting tubules and cells of collecting ducts of phosphorus-treated rats.

TABLE I.

Comparative Histochemistry of Acid Hydrolases in Homologous Tissues of Different Species.

Table I-1 to Table I-33 inclusive.

Table I-1	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Kidney, Proximal tubule, Proximal portion									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X						X
Northern Pike					X					X
Newt										
Salamander										
Grass Frog										X
Tree Frog					X					X
Turtle				X						X
Chamelion			X						X	
Chicken				X						X
Magpie				X						X
Robin					X					X
Horned Owl				X					X	
Jap. Quail					X					X
Rabbit					X				X	
Guinea Pig					X					X
Golden Hamster					X					X
Mouse (Ajax)					X					X
Field Mouse				X					X	
Rat					X					X
Cow					X				X	
Sheep					X					X
Horse					X					X
Pig					X					X
Cat			X					X		
Dog					X					X
Man (mature)			X				X			
Man (infant)										

Table I-1	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Kidney, Proximal Tubule, Proximal Portion									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X			X			
Northern Pike				X					X	
Newt										
Salamander										
Grass Frog			X							X
Tree Frog		X							X	
Turtle		X								X
Chamelion	X						X			
Chicken			X						X	
Magpie					X				X	
Robin					X					X
Horned Owl	X									X
Jap. Quail					X					X
Rabbit	X					X				
Guinea Pig	X									X
Golden Hamster		X					X			
Mouse (Ajax)					X		X			
Field Mouse	X							X		
Rat			X							X
Cow				X					X	
Sheep				X						X
Horse			X							X
Pig	X									X
Cat			X						X	
Dog					X					X
Man (mature)				X					X	
Man (infant)										

Table I-1	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Kidney, Proximal tubule, Proximal portion									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X						X
Northern Pike				X					X	
Newt										
Salamander										
Grass Frog					X					X
Tree Frog					X					X
Turtle					X			X		
Chamelion				X					X	
Chicken					X			X		
Magpie				X					X	
Robin					X			X		
Horned Owl					X				X	
Jap. Quail					X				X	
Rabbit					X					X
Guinea Pig					X					X
Golden Hamster					X					X
Mouse (Ajax)					X			X		
Field Mouse				X					X	
Rat					X					X
Cow					X					
Sheep					X				X	
Horse					X					X
Pig					X					X
Cat					X					X
Dog					X					X
Man (mature)				X						X
Man (infant)										

Table I-2	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Kidney, Proximal tubule, Mid portion									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X							X	
Northern Pike				X				X		
Newt										
Salamander										
Grass Frog										
Tree Frog			X						X	
Turtle		X							X	
Chamelion			X				X			
Chicken				X					X	
Magpie										
Robin			X						X	
Horned Owl										
Jap. Quail										
Rabbit										
Guinea Pig				X						
Golden Hamster										
Mouse (Ajax)				X						X
Field Mouse										
Rat										
Cow					X				X	
Sheep										
Horse										
Pig										
Cat										
Dog										
Man (mature)		X					X			
Man (infant)										

Table I-2	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Kidney, Proximal tubule, Mid portion									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X				X				
Northern Pike			X					X		
Newt										
Salamander										
Grass Frog										
Tree Frog		X						X		
Turtle	X								X	
Chamelion	X					X				
Chicken		X						X		
Magpie										
Robin		X					X			
Horned Owl										
Jap. Quail										
Rabbit										
Guinea Pig										
Golden Hamster								X		
Mouse (Ajax)					X		X			
Field Mouse	X									
Rat										
Cow				X					X	
Sheep										
Horse										
Pig										
Cat									X	
Dog										
Man (mature)			X					X		
Man (infant)										

Table I-2	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Kidney, Proximal tubule, Mid portion									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X					X			
Northern Pike			X					X		
Newt										
Salamander										
Grass Frog										
Tree Frog					X				X	
Turtle					X			X		
Chamelion				X			X			
Chicken					X		X			
Magpie										
Robin				X				X		
Horned Owl										
Jap. Quail										
Rabbit										
Guinea Pig										
Golden Hamster					X					
Mouse (Ajax)				X				X		
Field Mouse										
Rat					X					X
Cow					X					
Sheep										
Horse										
Pig										
Cat										
Dog										
Man (mature)				X				X		
Man (infant)										

Table I-3	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Kidney, Proximal tubule, Distal portion									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X					X			
Northern Pike				X					X	
Newt										
Salamander										
Grass Frog										
Tree Frog								X		
Turtle		X						X		
Chamelion										
Chicken		X						X		
Magpie										
Robin		X						X		
Horned Owl										
Jap. Quail								X		
Rabbit										
Guinea Pig				X					X	
Golden Hamster				X					X	
Mouse (Ajax)			X						X	
Field Mouse										
Rat				X					X	
Cow			X					X		
Sheep		X								
Horse								X		
Pig			X					X		
Cat										
Dog					X			X		
Man (mature)		X					X			
Man (infant)										

Table I-3	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Kidney, Proximal tubule, Distal portion									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike			X					X		
Newt										
Salamander										
Grass Frog										
Tree Frog		X					X			
Turtle	X								X	
Chamelion	X					X				
Chicken		X						X		
Magpie										
Robin		X					X			
Horned Owl										
Jap. Quail						X				
Rabbit										
Guinea Pig			X					X		
Golden Hamster		X					X			
Mouse (Ajax)				X				X		
Field Mouse	X									
Rat		X						X		
Cow		X					X			
Sheep										
Horse										
Pig										
Cat										
Dog			X							
Man (mature)			X				X			
Man (infant)										

Table I-3	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Kidney, Proximal tubule, Distal portion									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X					X			
Northern Pike			X					X		
Newt										
Salamander										
Grass Frog		X								
Tree Frog		X								
Turtle					X			X		
Chamelion				X						
Chicken					X		X			
Magpie										
Robin				X				X		
Horned Owl										
Jap. Quail										
Rabbit				X					X	
Guinea Pig				X						
Golden Hamster					X			X		
Mouse (Ajax)					X					X
Field Mouse				X				X		
Rat					X					X
Cow				X						
Sheep				X					X	
Horse					X			X		
Pig					X		X			
Cat				X					X	
Dog			X						X	
Man (mature)				X			X			
Man (infant)										

Table I-4	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Kidney, Distal tubule									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike										
Northern Pike										
Newt										
Salamander										
Grass Frog			X				X			
Tree Frog			X			X				
Turtle	X						X			
Chamelion	X					X				
Chicken		X					X			
Magpie		X					X			
Robin	X						X			
Horned Owl	X						X			
Jap. Quail		X				X				
Rabbit		X					X			
Guinea Pig	X					X				
Golden Hamster		X				X				
Mouse (Ajax)		X					X			
Field Mouse		X					X			
Rat		X				X				
Cow	X						X			
Sheep	X						X			
Horse		X				X				
Pig		X				X				
Cat		X					X			
Dog	X					X				
Man (mature)	X					X				
Man (infant)										

Table I-4	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Kidney, Distal tubule									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike										
Northern Pike										
Newt										
Salamander										
Grass Frog	X					X				
Tree Frog	X					X				
Turtle		X						X		
Chamelion	X					X				
Chicken		X					X			
Magpie	X					X				
Robin							X			
Horned Owl	X					X				
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X					X				
Mouse (Ajax)			X				X			
Field Mouse	X					X				
Rat	X					X				
Cow	X					X				
Sheep	X					X				
Horse		X				X				
Pig	X					X				
Cat	X					X				
Dog	X					X				
Man (mature)	X					X				
Man (infant)										

Table I-4	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Kidney, Distal tubule									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike										
Northern Pike										
Newt										
Salamander										
Grass Frog		X					X			
Tree Frog		X						X		
Turtle		X					X			
Chamelion				X		X				
Chicken	X					X				
Magpie	X					X				
Robin			X				X			
Horned Owl		X				X				
Jap. Quail	X						X			
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X					X				
Mouse (Ajax)	X						X			
Field Mouse	X					X				
Rat	X					X				
Cow	X									
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat	X					X				
Dog	X					X				
Man (mature)			X			X				
Man (infant)										

Table I-5	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Kidney, Loop of Henle									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike							X			
Northern Pike										
Newt										
Salamander										
Grass Frog		X								
Tree Frog		X					X			
Turtle	X						X			
Chamelion	X									
Chicken										
Magpie										
Robin		X					X			
Horned Owl										
Jap. Quail										
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster		X					X			
Mouse (Ajax)		X				X				
Field Mouse	X					X				
Rat		X					X			
Cow	X						X			
Sheep	X						X			
Horse	X					X				
Pig	X						X			
Cat	X					X				
Dog		X				X				
Man (mature)		X				X				
Man (infant)										

Table I-5	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Kidney, Loop of Henle									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike										
Northern Pike										
Newt										
Salamander										
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X						X			
Chamelion	X					X				
Chicken										
Magpie										
Robin	X					X				
Horned Owl										
Jap. Quail										
Rabbit	X					X				
Guinea Pig	X						X			
Golden Hamster	X						X			
Mouse (Ajax)	X					X				
Field Mouse	X					X				
Rat	X					X				
Cow	X					X				
Sheep	X					X				
Horse		X				X				
Pig		X				X				
Cat	X					X				
Dog	X					X				
Man (mature)	X					X				
Man (infant)										

Table I-5	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Kidney, Loop of Henle									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike										
Northern Pike										
Newt										
Salamander										
Grass Frog							X			
Tree Frog		X					X			
Turtle		X				X				
Chamelion	X					X				
Chicken										
Magpie										
Robin	X					X				
Horned Owl										
Jap. Quail										
Rabbit	X					X				
Guinea Pig		X					X			
Golden Hamster	X						X			
Mouse (Ajax)	X						X			
Field Mouse	X					X				
Rat	X						X			
Cow	X									
Sheep	X					X				
Horse		X					X			
Pig		X					X			
Cat	X					X				
Dog	X					X				
Man (mature)			X				X			
Man (infant)										

Table I-6	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Kidney, Collecting tubule principal cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X					X			
Northern Pike		X					X			
Newt										
Salamander										
Grass Frog		X					X			
Tree Frog		X					X			
Turtle			X					X		
Chamelion	X						X			
Chicken		X						X		
Magpie										
Robin		X					X			
Horned Owl						X				
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster			X				X			
Mouse (Ajax)		X					X			
Field Mouse						X				
Rat		X					X			
Cow	X					X				
Sheep	X					X				
Horse	X					X				
Pig				X		X				
Cat	X					X				
Dog	X					X				
Man (mature)	X					X				
Man (infant)										

Table I-6	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Kidney, Collecting tubule, Principal cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt										
Salamander										
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X							X		
Chamelion	X					X				
Chicken		X					X			
Magpie										
Robin	X						X			
Horned Owl	X					X				
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig		X				X				
Golden Hamster					X	X				
Mouse (Ajax)	X					X				
Field Mouse	X					X				
Rat	X					X				
Cow	X					X				
Sheep	X					X				
Horse	X					X				
Pig	X									
Cat	X					X				
Dog	X					X				
Man (mature)	X					X				
Man (infant)										

Table I-6	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Kidney, Collecting tubule, Principal cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X							X		
Northern Pike		X					X			
Newt										
Salamander										
Grass Frog	X						X			
Tree Frog			X							X
Turtle		X					X			
Chamelion	X					X				
Chicken	X					X				
Magpie										
Robin			X				X			
Horned Owl	X					X				
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X					X				
Mouse (Ajax)	X					X				
Field Mouse	X					X				
Rat	X					X				
Cow	X									
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat	X					X				
Dog	X					X				
Man (mature)	X					X				
Man (infant)										

Table I-7	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Kidney, Collecting tubule, Intercalated cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike										
Northern Pike										
Newt										
Salamander										
Grass Frog										
Tree Frog										
Turtle										
Chamelion										
Chicken				X				X		
Magpie	X									
Robin										
Horned Owl	X							X		
Jap. Quail		X						X		
Rabbit			X						X	
Guinea Pig				X				X		
Golden Hamster				X					X	
Mouse (Ajax)				X						X
Field Mouse								X		
Rat				X				X		
Cow			X						X	
Sheep		X								X
Horse			X						X	
Pig				X				X		
Cat	X								X	
Dog		X						X		
Man (mature)	X						X			
Man (infant)										

Table I-7	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Kidney, Collecting tubule, Intercalated cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike										
Northern Pike										
Newt										
Salamander										
Grass Frog										
Tree Frog										
Turtle										
Chamelion										
Chicken		X					X			
Magpie										
Robin										
Horned Owl	X						X			
Jap. Quail		X					X			
Rabbit		X				X				
Guinea Pig				X					X	
Golden Hamster					X				X	
Mouse (Ajax)			X							X
Field Mouse	X					X				
Rat		X						X		
Cow		X							X	
Sheep			X					X		
Horse			X					X		
Pig	X							X		
Cat	X									X
Dog			X				X			
Man (mature)		X					X			
Man (infant)										

Table I-7	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Kidney, Collecting tubule, Intercalated cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike										
Northern Pike										
Newt										
Salamander										
Grass Frog										
Tree Frog										
Turtle										
Chamelion										
Chicken	X					X				
Magpie										
Robin										
Horned Owl	X					X				
Jap. Quail	X					X				
Rabbit				X				X		
Guinea Pig		X					X			
Golden Hamster		X								X
Mouse (Ajax)				X			X			
Field Mouse			X					X		
Rat		X								X
Cow			X							
Sheep			X					X		
Horse			X							X
Pig				X				X		
Cat				X		X				
Dog	X						X			
Man (mature)				X						X
Man (infant)										

Table I-8	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Kidney, collecting ducts									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X								X	
Northern Pike		X						X		
Newt										
Salamander										
Grass Frog										
Tree Frog		X					X			
Turtle			X						X	
Chamelion	X						X			
Chicken										
Magpie		X					X			
Robin			X					X		
Horned Owl			X			X				
Jap. Quail		X					X			
Rabbit	X					X				
Guinea Pig		X				X				
Golden Hamster	X						X			
Mouse (Ajax)	X					X				
Field Mouse						X				
Rat	X						X			
Cow			X			X				
Sheep	X					X				
Horse		X				X				
Pig					X		X			
Cat	X					X				
Dog	X					X				
Man (mature)	X									
Man (infant)										

Table I-8	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Kidney, Collecting duct									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt										
Salamander										
Grass Frog										
Tree Frog	X					X				
Turtle	X							X		
Chamelion	X					X				
Chicken										
Magpie		X				X				
Robin	X									X
Horned Owl	X						X			
Jap. Quail			X				X			
Rabbit	X					X				
Guinea Pig			X				X			
Golden Hamster	X					X				
Mouse (Ajax)	X								X	
Field Mouse	X					X				
Rat	X								X	
Cow				X		X				
Sheep	X					X				
Horse		X				X				
Pig	X							X		
Cat	X								X	
Dog			X						X	
Man (mature)		X				X				
Man (infant)										

Table I-8	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Kidney, Collecting ducts									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X							X		
Northern Pike		X					X			
Newt										
Salamander										
Grass Frog										
Tree Frog		X					X			
Turtle		X					X			
Chamelion	X					X				
Chicken										
Magpie	X					X				
Robin				X				X		
Horned Owl		X					X			
Jap. Quail	X						X			
Rabbit		X					X			
Guinea Pig		X				X				
Golden Hamster					X		X			
Mouse (Ajax)		X					X			
Field Mouse	X					X				
Rat								X		
Cow	X									
Sheep	X					X				
Horse	X						X			
Pig	X						X			
Cat	X					X				
Dog	X						X			
Man (mature)			X				X			
Man (infant)										

Table I-9	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Kidney, Glomerulus, Visc. epithelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt										
Salamander										
Grass Frog	X					X				
Tree Frog		X					X			
Turtle	X						X			
Chamelion	X					X				
Chicken		X					X			
Magpie			X				X			
Robin		X				X				
Horned Owl	X					X				
Jap. Quail	X					X				
Rabbit	X						X			
Guinea Pig		X								
Golden Hamster	X						X			
Mouse (Ajax)			X				X			
Field Mouse	X					X				
Rat	X					X				
Cow				X		X				
Sheep		X					X			
Horse	X					X				
Pig	X					X				
Cat		X				X				
Dog			X			X				
Man (mature)	X					X				
Man (infant)										

Table I-9	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Kidney, Glomerulus, Visceral epithelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt										
Salamander										
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X					X				
Chamelion	X					X				
Chicken	X					X				
Magpie		X				X				
Robin	X					X				
Horned Owl	X					X				
Jap. Quail	X						X			
Rabbit	X					X				
Guinea Pig		X						X		
Golden Hamster		X				X				
Mouse (Ajax)	X					X				
Field Mouse	X					X				
Rat		X				X				
Cow		X					X			
Sheep	X					X				
Horse		X				X				
Pig	X					X				
Cat	X					X				
Dog	X					X				
Man (mature)	X					X				
Man (infant)										

Table I-9	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Kidney, Glomerulus, Visceral epithelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt										
Salamander										
Grass Frog	X					X				
Tree Frog		X					X			
Turtle	X					X				
Chamelion	X					X				
Chicken				X				X		
Magpie	X						X			
Robin	X								X	
Horned Owl	X					X				
Jap. Quail	X						X			
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X						X			
Mouse (Ajax)	X						X			
Field Mouse	X					X				
Rat	X						X			
Cow	X									
Sheep	X					X				
Horse		X					X			
Pig				X			X			
Cat	X					X				
Dog	X					X				
Man (mature)			X				X			
Man (infant)										

Table I-10	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Kidney, Glomerulus, Parietal epithelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt										
Salamander										
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X					X				
Chamelion	X					X				
Chicken	X					X				
Magpie	X					X				
Robin	X					X				
Horned Owl	X					X				
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X					X				
Mouse (Ajax)	X					X				
Field Mouse	X					X				
Rat	X					X				
Cow	X					X				
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat	X					X				
Dog	X					X				
Man (mature)	X					X				
Man (infant)										

Table I-10	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Kidney, Glomerulus, Parietal epithelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt										
Salamander										
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X						X			
Chamelion	X					X				
Chicken	X					X				
Magpie	X					X				
Robin	X					X				
Horned Owl	X					X				
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X					X				
Mouse (Ajax)	X					X				
Field Mouse	X					X				
Rat	X					X				
Cow	X					X				
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat	X					X				
Dog	X					X				
Man (mature)	X					X				
Man (infant)										

Table I-10	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Kidney, Glomerulus, Parietal epithelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt										
Salamander										
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X					X				
Chamelion	X					X				
Chicken	X					X				
Magpie	X					X				
Robin	X						X			
Horned Owl	X					X				
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X						X			
Mouse (Ajax)	X					X				
Field Mouse	X					X				
Rat	X					X				
Cow	X									
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat	X					X				
Dog	X					X				
Man (mature)	X					X				
Man (infant)										

Table I-11	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Kidney, Glomerulus, Endothelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X						X			
Northern Pike	X					X				
Newt										
Salamander										
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X					X				
Chamelion	X					X				
Chicken	X					X				
Magpie	X					X				
Robin	X					X				
Horned Owl	X					X				
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X						X			
Mouse (Ajax)	X					X				
Field Mouse	X					X				
Rat	X					X				
Cow	X					X				
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat	X					X				
Dog	X					X				
Man (mature)	X					X				
Man (infant)										

Table I-11	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Kidney, Glomerulus, Endothelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt										
Salamander										
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X					X				
Chamelion	X					X				
Chicken	X					X				
Magpie	X					X				
Robin	X					X				
Horned Owl	X					X				
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X					X				
Mouse (Ajax)	X					X				
Field Mouse	X					X				
Rat	X					X				
Cow	X					X				
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat	X					X				
Dog	X					X				
Man (mature)	X					X				
Man (infant)										

Table I-11	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Kidney, Glomerulus, Endothelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt										
Salamander										
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X					X				
Chamelion	X					X				
Chicken	X					X				
Magpie	X					X				
Robin	X					X				
Horned Owl	X					X				
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X						X			
Mouse (Ajax)	X					X				
Field Mouse	X					X				
Rat	X					X				
Cow	X									
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat	X					X				
Dog	X					X				
Man (mature)	X					X				
Man (infant)										

Table I-12	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Liver, Lobule, Central									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike			X						X	
Northern Pike					X					X
Newt										
Salamander									X	
Grass Frog					X				X	
Tree Frog				X					X	
Turtle			X					X		
Chamelion			X					X		
Chicken			X					X		
Magpie					X				X	
Robin			X					X		
Horned Owl				X					X	
Jap. Quail					X			X		
Rabbit			X					X		
Guinea Pig				X					X	
Golden Hamster				X				X		
Mouse (Ajax)			X					X		
Field Mouse	X							X		
Rat				X				X		
Cow		X					X			
Sheep	X						X			
Horse					X				X	
Pig					X				X	
Cat				X					X	
Dog			X					X		
Man (mature)			X					X		
Man (infant)				X					X	

Table I-12	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Liver, Lobule, Central									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X				X		
Northern Pike					X				X	
Newt				X						
Salamander		X							X	
Grass Frog			X				X			
Tree Frog			X			X				
Turtle				X				X		
Chamelion	X					X				
Chicken		X					X			
Magpie				X			X			
Robin		X					X			
Horned Owl	X						X			
Jap. Quail			X				X			
Rabbit				X		X				
Guinea Pig			X						X	
Golden Hamster			X			X				
Mouse (Ajax)		X				X				
Field Mouse		X				X				
Rat			X			X				
Cow		X				X				
Sheep			X			X				
Horse			X				X			
Pig		X					X			
Cat			X					X		
Dog		X					X			
Man (mature)		X					X			
Man (infant)				X			X			

Table I-12	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Liver, Lobule, Central									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X					X	
Northern Pike				X					X	
Newt										
Salamander		X				X				
Grass Frog				X					X	
Tree Frog					X					X
Turtle			X			X				
Chamelion				X					X	
Chicken				X				X		
Magpie					X				X	
Robin				X			X			
Horned Owl					X			X		
Jap. Quail					X				X	
Rabbit				X						
Guinea Pig				X					X	
Golden Hamster					X				X	
Mouse (Ajax)					X			X		
Field Mouse				X				X		
Rat					X				X	
Cow					X					
Sheep					X			X		
Horse					X					
Pig					X					
Cat					X			X		
Dog					X				X	
Man (mature)				X				X		
Man (infant)					X			X		

Table I-13	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Liver, Lobule, Peripheral									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike			X						X	
Northern Pike					X					X
Newt										
Salamander									X	
Grass Frog					X				X	
Tree Frog				X					X	
Turtle			X					X		
Chamelion			X					X		
Chicken			X					X		
Magpie					X					X
Robin			X					X		
Horned Owl				X					X	
Jap. Quail					X				X	
Rabbit				X					X	
Guinea Pig					X					X
Golden Hamster					X				X	
Mouse (Ajax)				X					X	
Field Mouse		X					X			
Rat					X				X	
Cow		X						X		
Sheep	X							X		
Horse				X						X
Pig				X				X		
Cat			X					X		
Dog				X					X	
Man (mature)			X					X		
Man (infant)					X					X

Table I-13	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Liver, Lobule, Peripheral									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X				X		
Northern Pike					X				X	
Newt				X						
Salamander		X							X	
Grass Frog			X				X			
Tree Frog			X			X				
Turtle				X				X		
Chamelion	X					X				
Chicken		X					X			
Magpie				X			X			
Robin		X						X		
Horned Owl	X						X			
Jap. Quail			X				X			
Rabbit					X	X				
Guinea Pig				X						X
Golden Hamster				X		X				
Mouse (Ajax)			X				X			
Field Mouse			X					X		
Rat				X		X				
Cow			X			X				
Sheep				X		X				
Horse				X			X			
Pig			X				X			
Cat				X				X		
Dog				X				X		
Man (mature)			X					X		
Man (infant)				X				X		

Table I-13	ENZYME: N Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Liver, Lobule, Peripheral									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike			X						X	
Northern Pike				X					X	
Newt										
Salamander		X				X				
Grass Frog				X					X	
Tree Frog										X
Turtle			X			X				
Chamelion				X					X	
Chicken					X				X	
Magpie					X				X	
Robin				X			X			
Horned Owl					X			X		
Jap. Quail					X				X	
Rabbit					X					
Guinea Pig				X					X	
Golden Hamster					X					X
Mouse (Ajax)					X				X	
Field Mouse				X				X		
Rat					X					X
Cow					X					X
Sheep					X				X	
Horse					X				X	
Pig					X					X
Cat				X						X
Dog					X					X
Man (mature)				X					X	
Man (infant)					X			X		

Table I-14	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Liver, Parenchymal cells, Pericanalicular									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X					X	
Northern Pike					X					X
Newt										
Salamander									X	
Grass Frog					X				X	
Tree Frog				X						X
Turtle			X					X		
Chamelion			X					X		
Chicken			X					X		
Magpie					X					X
Robin			X				X			
Horned Owl				X					X	
Jap. Quail					X			X		
Rabbit				X					X	
Guinea Pig					X				X	
Golden Hamster					X				X	
Mouse (Ajax)				X					X	
Field Mouse	X						X			
Rat					X				X	
Cow		X					X			
Sheep	X							X		
Horse					X					X
Pig					X				X	
Cat				X					X	
Dog				X					X	
Man (mature)			X					X		
Man (infant)				X						X

Table I-14	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Liver, Parenchymal cells, Pericanalicular									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X				X		
Northern Pike					X				X	
Newt				X						
Salamander				X						X
Grass Frog			X				X			
Tree Frog			X			X				
Turtle		X							X	
Chamelion	X					X				
Chicken		X				X				
Magpie				X			X			
Robin		X					X			
Horned Owl	X						X			
Jap. Quail			X				X			
Rabbit					X	X				
Guinea Pig					X					X
Golden Hamster			X			X				
Mouse (Ajax)			X			X				
Field Mouse		X				X				
Rat				X		X				
Cow				X		X				
Sheep				X		X				
Horse			X				X			
Pig		X					X			
Cat			X					X		
Dog		X						X		
Man (mature)				X				X		
Man (infant)					X		X			

Table I-14	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Liver, Parenchymal cells, Pericanalicular									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X						X
Northern Pike				X					X	
Newt										
Salamander		X					X			
Grass Frog				X					X	
Tree Frog					X				X	
Turtle			X			X				
Chamelion				X					X	
Chicken				X					X	
Magpie					X				X	
Robin				X			X			
Horned Owl					X			X		
Jap. Quail					X				X	
Rabbit				X						
Guinea Pig				X					X	
Golden Hamster					X				X	
Mouse (Ajax)					X				X	
Field Mouse				X				X		
Rat					X					X
Cow					X					X
Sheep					X				X	
Horse					X					X
Pig					X					X
Cat					X				X	
Dog					X					X
Man (mature)				X					X	
Man (infant)					X			X		

Table I-15	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Liver, Parenchymal cells, Perinuclear									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt										
Salamander						X				
Grass Frog	X						X			
Tree Frog		X								X
Turtle	X					X				
Chamelion		X					X			
Chicken	X					X				
Magpie	X					X				
Robin	X					X				
Horned Owl	X					X				
Jap. Quail		X				X				
Rabbit		X					X			
Guinea Pig	X							X		
Golden Hamster	X								X	
Mouse (Ajax)	X						X			
Field Mouse	X						X			
Rat	X						X			
Cow	X						X			
Sheep	X					X				
Horse	X						X			
Pig	X						X			
Cat	X						X			
Dog		X					X			
Man (mature)	X					X				
Man (infant)	X					X				

Table I-15	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Liver, Parenchymal cells, Perinuclear									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt		X								
Salamander	X					X				
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X					X				
Chamelion	X					X				
Chicken	X					X				
Magpie	X					X				
Robin	X					X				
Horned Owl	X					X				
Jap. Quail		X				X				
Rabbit		X				X				
Guinea Pig	X					X				
Golden Hamster		X				X				
Mouse (Ajax)		X				X				
Field Mouse	X					X				
Rat	X					X				
Cow			X			X				
Sheep	X					X				
Horse	X					X				
Pig						X				
Cat	X						X			
Dog		X					X			
Man (mature)	X					X				
Man (infant)	X					X				

Table I-15	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Liver, Parenchymal cells, Perinuclear									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X							X	
Northern Pike				X					X	
Newt										
Salamander	X					X				
Grass Frog		X								
Tree Frog					X				X	
Turtle			X			X				
Chamelion				X					X	
Chicken				X		X				
Magpie					X				X	
Robin				X			X			
Horned Owl				X				X		
Jap. Quail					X				X	
Rabbit				X						
Guinea Pig				X					X	
Golden Hamster	X							X		
Mouse (Ajax)					X				X	
Field Mouse				X				X		
Rat					X			X		
Cow					X					X
Sheep					X				X	
Horse			X						X	
Pig					X					X
Cat			X					X		
Dog					X					X
Man (mature)				X					X	
Man (infant)					X			X		

Table I-16	ENZYME: Acid Phosphatase (Comori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Liver, Kupffer cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X						X
Northern Pike					X					X
Newt										
Salamander										X
Grass Frog					X				X	
Tree Frog					X					X
Turtle	X							X		
Chamelion			X						X	
Chicken					X					X
Magpie					X					X
Robin					X					X
Horned Owl					X					X
Jap. Quail					X					X
Rabbit					X					X
Guinea Pig					X					X
Golden Hamster					X					X
Mouse (Ajax)					X					X
Field Mouse					X					X
Rat					X					X
Cow					X					X
Sheep		X							X	
Horse					X					X
Pig					X					X
Cat					X					X
Dog					X					X
Man (mature)				X				X		
Man (infant)					X					X

Table 1-16	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Liver, Kupffer cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X					X	
Northern Pike					X					X
Newt				X						
Salamander					X					X
Grass Frog					X			X		
Tree Frog				X				X		
Turtle					X					X
Chamelion		X				X				
Chicken		X							X	
Magpie					X				X	
Robin					X					X
Horned Owl	X									X
Jap. Quail				X						X
Rabbit					X			X		
Guinea Pig				X						X
Golden Hamster					X				X	
Mouse (Ajax)	X						X			
Field Mouse				X					X	
Rat					X		X			
Cow			X							X
Sheep					X				X	
Horse				X				X		
Pig		X								X
Cat					X					X
Dog					X					X
Man (mature)		X					X			
Man (infant)			X					X		

Table I-16	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Liver, Kupffer cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike			X						X	
Northern Pike				X				X		
Newt										
Salamander			X			X				
Grass Frog					X					X
Tree Frog					X					X
Turtle				X		X				
Chamelion		X				X				
Chicken				X				X		
Magpie					X				X	
Robin		X								X
Horned Owl		X								X
Jap. Quail		X						X		
Rabbit					X					
Guinea Pig				X						X
Golden Hamster					X				X	
Mouse (Ajax)					X				X	
Field Mouse				X				X		
Rat					X			X		
Cow					X					X
Sheep					X				X	
Horse				X					X	
Pig					X					X
Cat				X						X
Dog					X					X
Man (mature)				X						X
Man (infant)				X						X

Table I-17	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Liver, Bileduct epithelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X						X			
Northern Pike		X							X	
Newt										
Salamander						X				
Grass Frog	X									X
Tree Frog			X						X	
Turtle	X								X	
Chamelion		X				X				
Chicken		X					X			
Magpie		X							X	
Robin		X				X				
Horned Owl		X				X				
Jap. Quail	X					X				
Rabbit			X				X			
Guinea Pig		X				X				
Golden Hamster		X					X			
Mouse (Ajax)		X							X	
Field Mouse	X					X				
Rat		X					X			
Cow	X						X			
Sheep	X							X		
Horse		X					X			
Pig		X					X			
Cat		X					X			
Dog			X				X			
Man (mature)		X				X				
Man (infant)		X					X			

Table I-17	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Liver, Bileduct epithelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike		X				X				
Newt	X									
Salamander	X					X				
Grass Frog		X						X		
Tree Frog	X					X				
Turtle	X							X		
Chamelion	X					X				
Chicken	X					X				
Magpie				X		X				
Robin	X					X				
Horned Owl	X					X				
Jap. Quail		X				X				
Rabbit	X					X				
Guinea Pig					X					X
Golden Hamster	X						X			
Mouse (Ajax)			X			X				
Field Mouse	X					X				
Rat		X				X				
Cow	X					X				
Sheep	X						X			
Horse	X					X				
Pig	X					X				
Cat	X						X			
Dog				X					X	
Man (mature)		X					X			
Man (infant)		X							X	

Table I-17	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Liver, Bileduct epithelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X						X			
Northern Pike	X						X			
Newt										
Salamander	X					X				
Grass Frog					X					X
Tree Frog				X				X		
Turtle			X			X				
Chamelion			X					X		
Chicken	X					X				
Magpie		X					X			
Robin	X					X				
Horned Owl		X					X			
Jap. Quail	X						X			
Rabbit		X								
Guinea Pig		X							X	
Golden Hamster	X					X				
Mouse (Ajax)					X		X			
Field Mouse	X						X			
Rat			X				X			
Cow	X					X				
Sheep	X					X				
Horse			X							X
Pig				X				X		
Cat		X					X			
Dog			X				X			
Man (mature)			X				X			
Man (infant)			X				X			

Table I-18	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Pancreas, Islet cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X					X			
Northern Pike				X						X
Newt										
Salamander		X					X			
Grass Frog		X						X		
Tree Frog				X					X	
Turtle		X						X		
Chamelion	X					X				
Chicken		X							X	
Magpie		X					X			
Robin		X					X			
Horned Owl		X					X			
Jap. Quail		X						X		
Rabbit		X					X			
Guinea Pig		X					X			
Golden Hamster		X					X			
Mouse (Ajax)			X					X		
Field Mouse			X				X			
Rat			X					X		
Cow	X						X			
Sheep	X						X			
Horse	X						X			
Pig		X					X			
Cat			X				X			
Dog		X					X			
Man (mature)			X					X		
Man (infant)			X						X	

Table I-18	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Pancreas, Islet cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X								X	
Northern Pike	X								X	
Newt										
Salamander	X							X		
Grass Frog	X					X				
Tree Frog	X						X			
Turtle		X							X	
Chamelion	X						X			
Chicken	X						X			
Magpie		X				X				
Robin		X							X	
Horned Owl	X					X				
Jap. Quail	X						X			
Rabbit	X						X			
Guinea Pig	X					X				
Golden Hamster	X					X				
Mouse (Ajax)		X					X			
Field Mouse	X						X			
Rat		X						X		
Cow	X							X		
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat		X						X		
Dog	X						X			
Man (mature)	X					X				
Man (infant)	X					X				

Table I-18	ENZYME: N Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Pancreas, Islet cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X							X		
Northern Pike				X						X
Newt										
Salamander		X					X			
Grass Frog			X						X	
Tree Frog	X						X			
Turtle		X				X				
Chamelion	X						X			
Chicken	X						X			
Magpie		X					X			
Robin		X					X			
Horned Owl	X						X			
Jap. Quail		X						X		
Rabbit	X						X			
Guinea Pig		X					X			
Golden Hamster	X						X			
Mouse (Ajax)		X					X			
Field Mouse	X						X			
Rat		X						X		
Cow		X					X			
Sheep		X					X			
Horse		X					X			
Pig		X					X			
Cat	X						X			
Dog		X					X			
Man (mature)		X					X			
Man (infant)		X					X			

Table I-19	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Pancreas, Acinar cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X					X			
Northern Pike		X						X		
Newt										
Salamander		X						X		
Grass Frog		X						X		
Tree Frog			X				X			
Turtle		X					X			
Chamelion		X					X			
Chicken					X					X
Magpie		X					X			
Robin				X						X
Horned Owl		X					X			
Jap. Quail				X					X	
Rabbit		X					X			
Guinea Pig		X					X			
Golden Hamster			X				X			
Mouse (Ajax)			X				X			
Field Mouse		X					X			
Rat		X					X			
Cow			X				X			
Sheep		X					X			
Horse				X			X			
Pig		X					X			
Cat		X					X			
Dog		X					X			
Man (mature)					X			X		
Man (infant)					X					X

Table I-19	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Pancreas, Acinar cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt										
Salamander	X							X		
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X					X				
Chamelion	X						X			
Chicken		X				X				
Magpie	X					X				
Robin	X					X				
Horned Owl	X					X				
Jap. Quail	X						X			
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X					X				
Mouse (Ajax)	X					X				
Field Mouse	X					X				
Rat	X					X				
Cow	X					X				
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat	X					X				
Dog	X					X				
Man (mature)	X					X				
Man (infant)				X		X				

Table I-19	ENZYME: N Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Pancreas, Acinar cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X					X	
Northern Pike		X						X		
Newt										
Salamander		X						X		
Grass Frog			X						X	
Tree Frog			X						X	
Turtle				X				X		
Chamelion		X						X		
Chicken				X			X			
Magpie		X					X			
Robin			X					X		
Horned Owl			X				X			
Jap. Quail			X					X		
Rabbit		X					X			
Guinea Pig			X					X		
Golden Hamster			X					X		
Mouse (Ajax)			X						X	
Field Mouse			X					X		
Rat			X				X			
Cow		X							X	
Sheep			X					X		
Horse				X				X		
Pig		X							X	
Cat		X						X		
Dog				X				X		
Man (mature)					X					X
Man (infant)					X					X

Table I-20	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Pancreas, Duct epithelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike			X					X		
Northern Pike		X						X		
Newt										
Salamander	X						X			
Grass Frog			X				X			
Tree Frog		X						X		
Turtle	X						X			
Chamelion	X					X				
Chicken				X						X
Magpie		X				X				
Robin	X						X			
Horned Owl	X					X				
Jap. Quail		X					X			
Rabbit	X					X				
Guinea Pig		X					X			
Golden Hamster		X					X			
Mouse (Ajax)		X					X			
Field Mouse	X					X				
Rat	X						X			
Cow		X					X			
Sheep	X					X				
Horse			X			X				
Pig	X						X			
Cat	X					X				
Dog		X						X		
Man (mature)		X					X			
Man (infant)		X					X			

Table I-20	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Pancreas, Duct epithelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X						X			
Northern Pike	X					X				
Newt										
Salamander	X					X				
Grass Frog					X					X
Tree Frog	X					X				
Turtle		X					X			
Chamelion	X					X				
Chicken			X						X	
Magpie	X					X				
Robin		X				X				
Horned Owl	X					X				
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig		X					X			
Golden Hamster	X					X				
Mouse (Ajax)	X					X				
Field Mouse	X					X				
Rat		X					X			
Cow		X				X				
Sheep	X					X				
Horse		X					X			
Pig	X						X			
Cat	X							X		
Dog			X					X		
Man (mature)	X						X			
Man (infant)			X				X			

Table I-20	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Pancreas, Duct epithelium									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X							X	
Northern Pike	X					X				
Newt										
Salamander		X					X			
Grass Frog			X							X
Tree Frog		X					X			
Turtle		X				X				
Chamelion	X						X			
Chicken					X			X		
Magpie		X					X			
Robin		X					X			
Horned Owl	X						X			
Jap. Quail	X					X				
Rabbit			X						X	
Guinea Pig	X							X		
Golden Hamster	X						X			
Mouse (Ajax)					X		X			
Field Mouse	X						X			
Rat	X						X			
Cow	X							X		
Sheep	X						X			
Horse			X						X	
Pig	X							X		
Cat					X		X			
Dog		X					X			
Man (mature)					X					X
Man (infant)					X					X

Table I-21	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Spleen, Red pulp, Macrophages									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X						X
Northern Pike			X							X
Newt					X				X	
Salamander				X						X
Grass Frog				X						X
Tree Frog								X		
Turtle				X					X	
Chamelion	X						X			
Chicken					X					X
Magpie					X				X	
Robin					X					X
Horned Owl				X				X		
Jap. Quail										
Rabbit					X					X
Guinea Pig					X					X
Golden Hamster	X							X		
Mouse (Ajax)					X					X
Field Mouse					X					X
Rat					X					X
Cow					X					X
Sheep					X					X
Horse					X					X
Pig					X					X
Cat					X					X
Dog										
Man (mature)				X				X		
Man (infant)				X					X	

Table I-21	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Spleen, Red pulp, Macrophages									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike					X					X
Northern Pike			X						X	
Newt		X					X			
Salamander			X							X
Grass Frog				X			X			
Tree Frog										
Turtle		X							X	
Chamelion	X						X			
Chicken				X						X
Magpie				X						
Robin				X					X	
Horned Owl	X									X
Jap. Quail							X			
Rabbit					X	X				
Guinea Pig					X					X
Golden Hamster				X						X
Mouse (Ajax)					X				X	
Field Mouse				X						X
Rat					X		X			
Cow					X				X	
Sheep					X				X	
Horse				X						X
Pig			X					X		
Cat					X			X		
Dog										
Man (mature)				X		X				
Man (infant)				X				X		

Table I-21	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Spleen, Red pulp, Macrophages									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X					X	
Northern Pike				X						X
Newt	X					X				
Salamander		X					X			
Grass Frog				X					X	
Tree Frog					X		X			
Turtle										
Chamelion				X			X			
Chicken		X				X				
Magpie		X					X			
Robin		X					X			
Horned Owl	X					X				
Jap. Quail										
Rabbit			X					X		
Guinea Pig			X						X	
Golden Hamster		X								X
Mouse (Ajax)				X						X
Field Mouse					X					X
Rat			X							X
Cow			X						X	
Sheep			X			X				
Horse			X					X		
Pig			X					X		
Cat		X						X		
Dog										
Man (mature)				X				X		
Man (infant)			X				X			

Table I-22	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Spleen, Red pulp, Cords of Billroth									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X					X			
Northern Pike		X						X		
Newt	X							X		
Salamander	X							X		
Grass Frog		X						X		
Tree Frog							X			
Turtle	X						X			
Chamelion	X						X			
Chicken		X					X			
Magpie		X					X			
Robin			X					X		
Horned Owl		X					X			
Jap. Quail										
Rabbit		X								
Guinea Pig		X					X			
Golden Hamster		X				X				
Mouse (Ajax)	X						X			
Field Mouse		X						X		
Rat		X						X		
Cow				X		X				
Sheep		X					X			
Horse	X					X				
Pig	X						X			
Cat		X					X			
Dog										
Man (mature)	X					X				
Man (infant)		X						X		

Table I-22	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Spleen, Red Pulp, Cords of Billroth									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X						X			
Northern Pike	X					X				
Newt	X					X				
Salamander	X					X				
Grass Frog		X				X				
Tree Frog										
Turtle	X					X				
Chamelion	X						X			
Chicken	X						X			
Magpie	X					X				
Robin		X				X				
Horned Owl	X					X				
Jap. Quail										
Rabbit		X					X			
Guinea Pig		X					X			
Golden Hamster	X						X			
Mouse (Ajax)		X				X				
Field Mouse	X					X				
Rat		X				X				
Cow		X					X			
Sheep	X							X		
Horse	X					X				
Pig	X					X				
Cat			X				X			
Dog										
Man (mature)	X					X				
Man (infant)		X				X				

Table I-22	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Spleen, Red pulp, Cords of Billroth									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X						X			
Northern Pike		X					X			
Newt		X				X				
Salamander	X					X				
Grass Frog	X					X				
Tree Frog										
Turtle	X					X				
Chamelion		X				X				
Chicken	X					X				
Magpie	X					X				
Robin	X					X				
Horned Owl	X					X				
Jap. Quail										
Rabbit		X					X			
Guinea Pig	X						X			
Golden Hamster		X					X			
Mouse (Ajax)	X						X			
Field Mouse	X						X			
Rat	X						X			
Cow	X						X			
Sheep		X				X				
Horse			X				X			
Pig	X					X				
Cat	X					X				
Dog										
Man (mature)	X					X				
Man (infant)		X				X				

Table I-23	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Spleen, White pulp, Macrophages									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X						X
Northern Pike			X							X
Newt					X					X
Salamander				X						X
Grass Frog				X						X
Tree Frog								X		
Turtle				X					X	
Chamelion	X						X			
Chicken				X						X
Magpie					X				X	
Robin					X					X
Horned Owl				X					X	
Jap. Quail										
Rabbit					X	X				
Guinea Pig				X						X
Golden Hamster					X					X
Mouse (Ajax)					X					X
Field Mouse					X					X
Rat				X						X
Cow					X				X	
Sheep	X						X			
Horse					X					X
Pig					X			X		
Cat		X					X			
Dog										
Man (mature)			X					X		
Man (infant)				X					X	

Table I-23	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Spleen, White Pulp, Macrophages									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike					X					X
Northern Pike			X						X	
Newt			X						X	
Salamander		X								X
Grass Frog					X		X			
Tree Frog										
Turtle			X							X
Chamelion	X						X			
Chicken		X							X	
Magpie				X			X			
Robin		X					X			
Horned Owl	X						X			
Jap. Quail										
Rabbit		X				X				
Guinea Pig					X					X
Golden Hamster				X						X
Mouse (Ajax)					X					X
Field Mouse		X								X
Rat					X			X		
Cow		X					X			
Sheep	X					X				
Horse				X		X				
Pig			X						X	
Cat					X				X	
Dog										
Man (mature)				X		X				
Man (infant)			X					X		

Table I-23	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Spleen, White pulp, Macrophages									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X					X	
Northern Pike				X						X
Newt	X						X			
Salamander	X						X			
Grass Frog			X						X	
Tree Frog		X					X			
Turtle		X					X			
Chamelion			X				X			
Chicken	X					X				
Magpie		X					X			
Robin	X						X			
Horned Owl		X				X				
Jap. Quail										
Rabbit		X						X		
Guinea Pig			X						X	
Golden Hamster		X								X
Mouse (Ajax)		X							X	
Field Mouse		X						X		
Rat		X								
Cow		X							X	
Sheep	X						X			
Horse					X					X
Pig		X						X		
Cat		X						X		
Dog										
Man (mature)			X				X			
Man (infant)		X					X			

Table I-24	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Spleen, White pulp, Lymphoid cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X						X		
Northern Pike		X						X		
Newt	X								X	
Salamander	X							X		
Grass Frog		X					X			
Tree Frog							X			
Turtle	X						X			
Chamelion	X						X			
Chicken		X						X		
Magpie	X						X			
Robin	X							X		
Horned Owl		X				X				
Jap. Quail										
Rabbit		X				X				
Guinea Pig		X				X				
Golden Hamster		X					X			
Mouse (Ajax)	X						X			
Field Mouse	X						X			
Rat		X						X		
Cow	X						X			
Sheep	X					X				
Horse	X									
Pig	X							X		
Cat			X			X				
Dog										
Man (mature)	X					X				
Man (infant)		X				X				

Table I-24	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Spleen, White pulp, Lymphoid cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X						X			
Northern Pike	X					X				
Newt	X					X				
Salamander	X						X			
Grass Frog	X					X				
Tree Frog										
Turtle	X						X			
Chamelion	X						X			
Chicken	X					X				
Magpie	X					X				
Robin	X								X	
Horned Owl	X					X				
Jap. Quail										
Rabbit		X				X				
Guinea Pig		X					X			
Golden Hamster	X					X				
Mouse (Ajax)		X				X				
Field Mouse	X					X				
Rat		X				X				
Cow	X						X			
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat		X						X		
Dog						X				
Man (mature)	X					X				
Man (infant)	X					X				

Table I-24	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Spleen, White Pulp, Lymphoid Cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X						X			
Northern Pike							X			
Newt	X						X			
Salamander	X					X				
Grass Frog		X					X			
Tree Frog										
Turtle	X					X				
Chamelion		X				X				
Chicken	X					X				
Magpie	X					X				
Robin	X					X				
Horned Owl	X						X			
Jap. Quail										
Rabbit		X					X			
Guinea Pig	X						X			
Golden Hamster	X						X			
Mouse (Ajax)	X						X			
Field Mouse	X						X			
Rat	X									
Cow		X					X			
Sheep	X					X				
Horse	X						X			
Pig		X					X			
Cat	X						X			
Dog										
Man (mature)	X					X				
Man (infant)	X					X				

Table I-25	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Duodenum, Villus, epithelium, Apex									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X							X	
Northern Pike					X					X
Newt				X					X	
Salamander		X							X	
Grass Frog		X								X
Tree Frog					X					X
Turtle										
Chamelion					X					X
Chicken				X					X	
Magpie				X				X		
Robin				X						X
Horned Owl			X				X			
Jap. Quail					X				X	
Rabbit				X			X			
Guinea Pig				X					X	
Golden Hamster					X				X	
Mouse (Ajax)			X							X
Field Mouse		X					X			
Rat				X				X		
Cow					X				X	
Sheep										
Horse										X
Pig				X				X		
Cat		X						X		
Dog		X					X			
Man (mature)						X				
Man (infant)			X					X		

Table I-25	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Duodenum, Villus, epithelium, Apex									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X					X			
Northern Pike	X								X	
Newt			X						X	
Salamander	X								X	
Grass Frog		X								X
Tree Frog		X							X	
Turtle										
Chamelion	X						X			
Chicken		X						X		
Magpie		X				X				
Robin		X					X			
Horned Owl	X						X			
Jap. Quail					X					X
Rabbit		X				X				
Guinea Pig		X						X		
Golden Hamster		X						X		
Mouse (Ajax)		X					X			
Field Mouse		X					X			
Rat	X						X			
Cow	X					X				
Sheep										
Horse		X					X			
Pig	X					X				
Cat	X								X	
Dog	X						X			
Man (mature)	X					X				
Man (infant)	X					X				

Table I-25	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Duodenum, Villus, epithelium, Apex									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X						X
Northern Pike					X				X	
Newt									X	
Salamander					X				X	
Grass Frog					X					X
Tree Frog				X						X
Turtle										
Chamelion					X				X	
Chicken					X					X
Magpie					X				X	
Robin					X			X		
Horned Owl					X			X		
Jap. Quail					X					X
Rabbit					X				X	
Guinea Pig					X					X
Golden Hamster					X					X
Mouse (Ajax)					X				X	
Field Mouse					X				X	
Rat					X			X		
Cow					X				X	
Sheep					X			X		
Horse					X					X
Pig					X				X	
Cat	X						X			
Dog		X					X			
Man (mature)										
Man (infant)					X				X	

Table I-26	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Duodenum, Villus, epithelium, Middle									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X							X	
Northern Pike					X					X
Newt				X					X	
Salamander		X							X	
Grass Frog		X								X
Tree Frog					X					X
Turtle										
Chamelion					X					X
Chicken				X					X	
Magpie				X				X		
Robin				X						X
Horned Owl			X				X			
Jap. Quail					X				X	
Rabbit				X			X			
Guinea Pig			X					X		
Golden Hamster			X					X		
Mouse (Ajax)			X							X
Field Mouse		X					X			
Rat			X					X		
Cow				X					X	
Sheep										
Horse										X
Pig					X			X		
Cat		X						X		
Dog		X					X			
Man (mature)						X				
Man (infant)			X					X		

Table I-26	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Duodenum, Villus, epithelium, Middle									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X					X			
Northern Pike	X								X	
Newt			X						X	
Salamander	X								X	
Grass Frog		X								X
Tree Frog		X							X	
Turtle										
Chamelion	X						X			
Chicken		X						X		
Magpie		X				X				
Robin		X					X			
Horned Owl	X						X			
Jap. Quail					X					X
Rabbit		X				X				
Guinea Pig		X						X		
Golden Hamster		X						X		
Mouse (Ajax)		X					X			
Field Mouse		X					X			
Rat	X						X			
Cow	X					X				
Sheep										
Horse		X					X			
Pig	X					X				
Cat	X								X	
Dog	X						X			
Man (mature)	X					X				
Man (infant)	X					X				

Table I-26	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Duodenum, Villus, epithelium, Middle									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike				X					X	
Northern Pike			X						X	
Newt									X	
Salamander				X					X	
Grass Frog					X					X
Tree Frog				X						X
Turtle										
Chamelion					X				X	
Chicken					X					X
Magpie					X				X	
Robin				X				X		
Horned Owl					X			X		
Jap. Quail					X					X
Rabbit					X				X	
Guinea Pig					X				X	
Golden Hamster					X					X
Mouse (Ajax)					X					X
Field Mouse					X				X	
Rat					X			X		
Cow					X				X	
Sheep	X						X			
Horse	X									X
Pig				X				X		
Cat	X						X			
Dog		X					X			
Man (mature)										
Man (infant)					X				X	

Table I-27	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Duodenum, Villus,epithelium, Crypt									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X							X	
Northern Pike			X							X
Newt			X						X	
Salamander		X							X	
Grass Frog		X								X
Tree Frog				X					X	
Turtle										
Chamelion					X			X		
Chicken			X					X		
Magpie				X				X		
Robin			X							X
Horned Owl			X				X			
Jap. Quail					X			X		
Rabbit				X			X			
Guinea Pig		X						X		
Golden Hamster		X					X			
Mouse (Ajax)		X								X
Field Mouse		X					X			
Rat		X						X		
Cow				X					X	
Sheep										
Horse										X
Pig			X					X		
Cat		X						X		
Dog		X					X			
Man (mature)						X				
Man (infant)		X					X			

Table I-27	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Duodenum, Villus,epithelium, Crypt									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X				X				
Northern Pike	X							X		
Newt		X					X			
Salamander	X								X	
Grass Frog		X							X	
Tree Frog		X					X			
Turtle										
Chamelion	X						X			
Chicken		X						X		
Magpie		X				X				
Robin		X					X			
Horned Owl	X						X			
Jap. Quail		X					X			
Rabbit		X				X				
Guinea Pig		X					X			
Golden Hamster		X						X		
Mouse (Ajax)		X					X			
Field Mouse	X						X			
Rat	X						X			
Cow	X					X				
Sheep										
Horse		X				X				
Pig	X					X				
Cat	X								X	
Dog	X						X			
Man (mature)	X					X				
Man (infant)	X					X				

Table I-27	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Duodenum, Villus, epithelium, Crypt									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike			X						X	
Northern Pike			X						X	
Newt									X	
Salamander				X					X	
Grass Frog					X			X		
Tree Frog					X				X	
Turtle										
Chamelion				X					X	
Chicken					X					X
Magpie					X				X	
Robin				X				X		
Horned Owl					X		X			
Jap. Quail					X					X
Rabbit				X					X	
Guinea Pig					X				X	
Golden Hamster					X				X	
Mouse (Ajax)					X				X	
Field Mouse					X				X	
Rat					X			X		
Cow					X				X	
Sheep	X						X			
Horse					X					X
Pig				X				X		
Cat	X						X			
Dog		X					X			
Man (mature)										
Man (infant)					X				X	

Table I-28	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Duodenum, Lamina Propria Macrophage									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike					X					X
Northern Pike				X						X
Newt				X					X	
Salamander	X									X
Grass Frog				X				X		
Tree Frog					X					X
Turtle										
Chamelion		X								X
Chicken					X					X
Magpie				X						X
Robin		X								X
Horned Owl			X			X				
Jap. Quail					X					X
Rabbit				X			X			
Guinea Pig					X					X
Golden Hamster					X					X
Mouse (Ajax)					X					X
Field Mouse				X					X	
Rat					X					X
Cow					X					X
Sheep										
Horse										X
Pig					X				X	
Cat	X								X	
Dog		X							X	
Man (mature)						X				
Man (infant)		X					X			

Table I-28	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Duodenum, Lamina Propria Macrophages									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X						X			
Northern Pike			X							X
Newt			X							X
Salamander	X									X
Grass Frog				X				X		
Tree Frog				X						X
Turtle										
Chamelion		X				X				
Chicken		X								X
Magpie					X			X		
Robin		X								
Horned Owl	X							X		
Jap. Quail				X						X
Rabbit			X				X			
Guinea Pig					X					X
Golden Hamster			X							X
Mouse (Ajax)				X			X			
Field Mouse					X					X
Rat				X				X		
Cow					X					X
Sheep										
Horse				X					X	
Pig				X					X	
Cat					X				X	
Dog				X						X
Man (mature)		X					X			
Man (infant)		X					X			

Table I-28	ENZYME: N Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Duodenum, Lamina propria macrophages									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike					X					X
Northern Pike		X							X	
Newt						X				
Salamander		X					X			
Grass Frog		X					X			
Tree Frog					X					X
Turtle			X							
Chamelion		X				X				
Chicken		X				X				
Magpie					X		X			
Robin		X					X			
Horned Owl			X				X			
Jap. Quail	X						X			
Rabbit		X						X		
Guinea Pig	X					X				
Golden Hamster	X						X			
Mouse (Ajax)		X						X		
Field Mouse	X									X
Rat	X							X		
Cow		X								X
Sheep		X					X			
Horse					X					X
Pig	X							X		
Cat			X				X			
Dog		X					X			
Man (mature)										
Man (infant)									X	

Table I-29	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Brain Neurones Cortical									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X							X		
Northern Pike					X				X	
Newt	X					X				
Salamander					X					X
Grass Frog					X				X	
Tree Frog		X					X			
Turtle				X						X
Chamelion	X					X				
Chicken			X					X		
Magpie										
Robin				X				X		
Horned Owl										
Jap. Quail			X					X		
Rabbit					X			X		
Guinea Pig				X				X		
Golden Hamster					X				X	
Mouse (Ajax)					X					X
Field Mouse										
Rat		X						X		
Cow		X						X		
Sheep	X						X			
Horse		X				X				
Pig				X			X			
Cat		X						X		
Dog										
Man (mature)										
Man (infant)			X						X	

Table I-29	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Brain, Neurones, Cortical									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt				X						X
Salamander	X									X
Grass Frog	X					X				
Tree Frog	X					X				
Turtle		X								X
Chamelion	X					X				
Chicken		X					X			
Magpie										
Robin	X						X			
Horned Owl										
Jap. Quail	X					X				
Rabbit	X						X			
Guinea Pig	X									X
Golden Hamster		X				X				
Mouse (Ajax)				X			X			
Field Mouse										
Rat	X						X			
Cow	X									
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat		X					X			
Dog										
Man (mature)										
Man (infant)	X					X				

Table I-29	ENZYME: N Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Brain, Neurones, Cortical									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike		X								
Northern Pike	X						X			
Newt	X						X			
Salamander	X							X		
Grass Frog	X						X			
Tree Frog			X							X
Turtle						X				
Chamelion	X					X				
Chicken			X			X				
Magpie										
Robin		X					X			
Horned Owl										
Jap. Quail	X					X				
Rabbit		X					X			
Guinea Pig	X					X				
Golden Hamster		X							X	
Mouse (Ajax)		X					X			
Field Mouse										
Rat	X									X
Cow			X							X
Sheep			X			X				
Horse				X				X		
Pig	X							X		
Cat			X				X			
Dog										
Man (mature)			X							
Man (infant)	X						X			

Table I-30	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Brain, Neurones, Purkinje									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X						X			
Northern Pike		X						X		
Newt	X					X				
Salamander	X					X				
Grass Frog							X			
Tree Frog	X						X			
Turtle	X					X				
Chamelion	X					X				
Chicken		X						X		
Magpie										
Robin	X					X				
Horned Owl										
Jap. Quail		X					X			
Rabbit			X				X			
Guinea Pig		X						X		
Golden Hamster				X				X		
Mouse (Ajax)					X				X	
Field Mouse										
Rat		X						X		
Cow		X						X		
Sheep	X					X				
Horse			X			X				
Pig		X					X			
Cat		X					X			
Dog										
Man (mature)										
Man (infant)			X					X		

Table I-30	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Brain, Neurones, Purkinje									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt	X					X				
Salamander	X					X				
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X									
Chamelion	X					X				
Chicken		X					X			
Magpie										
Robin	X					X				
Horned Owl										
Jap. Quail	X					X				
Rabbit	X						X			
Guinea Pig	X							X		
Golden Hamster	X					X				
Mouse (Ajax)	X					X				
Field Mouse										
Rat	X						X			
Cow	X									
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat		X					X			
Dog										
Man (mature)						X				
Man (infant)	X					X				

Table I-30	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Brain, Neurones, Purkinje									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X									
Northern Pike	X					X				
Newt	X					X				
Salamander	X					X				
Grass Frog	X					X				
Tree Frog	X							X		
Turtle	X					X				
Chamelion	X					X				
Chicken			X			X				
Magpie										
Robin		X							X	
Horned Owl										
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster		X					X			
Mouse (Ajax)		X					X			
Field Mouse										
Rat			X				X			
Cow			X				X			
Sheep		X								
Horse				X				X		
Pig		X					X			
Cat			X				X			
Dog										
Man (mature)										
Man (infant)			X			X				

Table I-31	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Brain, Glial cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X									X
Newt	X					X				
Salamander	X					X				
Grass Frog				X			X			
Tree Frog	X						X			
Turtle	X								X	
Chamelion	X					X				
Chicken		X						X		
Magpie										
Robin		X				X				
Horned Owl										
Jap. Quail		X						X		
Rabbit		X				X				
Guinea Pig			X				X			
Golden Hamster			X					X		
Mouse (Ajax)			X				X			
Field Mouse										
Rat		X					X			
Cow		X							X	
Sheep	X					X				
Horse	X						X			
Pig			X				X			
Cat		X				X				
Dog										
Man (mature)										
Man (infant)			X					X		

Table I-31	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Brain, Glial cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt	X					X				
Salamander	X					X				
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X							X		
Chamelion	X					X				
Chicken	X					X				
Magpie										
Robin	X					X				
Horned Owl										
Jap. Quail	X					X				
Rabbit	X									X
Guinea Pig	X								X	
Golden Hamster	X					X				
Mouse (Ajax)	X					X				
Field Mouse										
Rat	X							X		
Cow	X									
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat		X					X			
Dog										
Man (mature)										
Man (infant)	X					X				

Table I-31	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Brain, Glial cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X									
Northern Pike		X						X		
Newt	X					X				
Salamander	X					X				
Grass Frog	X					X				
Tree Frog	X						X			
Turtle	X					X				
Chamelion	X					X				
Chicken		X				X				
Magpie										
Robin	X					X				
Horned Owl										
Jap. Quail	X						X			
Rabbit	X					X				
Guinea Pig	X						X			
Golden Hamster	X						X			
Mouse (Ajax)	X					X				
Field Mouse										
Rat		X					X			
Cow	X						X			
Sheep		X				X				
Horse		X					X			
Pig	X							X		
Cat	X					X				
Dog										
Man (mature)										
Man (infant)	X					X				

Table I-32	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Brain, Endothelial cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike		X					X			
Newt	X					X				
Salamander	X					X				
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X					X				
Chamelion	X					X				
Chicken		X					X			
Magpie										
Robin		X				X				
Horned Owl										
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X					X				
Mouse (Ajax)	X					X				
Field Mouse										
Rat	X					X				
Cow	X					X				
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat	X					X				
Dog										
Man (mature)										
Man (infant)	X					X				

Table I-32	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Brain, Endothelial cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike	X					X				
Newt	X					X				
Salamander	X					X				
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X					X				
Chamelion	X					X				
Chicken	X					X				
Magpie										
Robin	X					X				
Horned Owl										
Jap. Quail	X					X				
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X					X				
Mouse (Ajax)	X					X				
Field Mouse										
Rat	X					X				
Cow	X									
Sheep	X					X				
Horse	X					X				
Pig	X					X				
Cat	X					X				
Dog										
Man (mature)										
Man (infant)	X					X				

Table I-32	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Brain, Endothelial cells									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X									
Northern Pike	X						X			
Newt	X					X				
Salamander	X					X				
Grass Frog	X					X				
Tree Frog	X					X				
Turtle	X					X				
Chamelion	X					X				
Chicken	X					X				
Magpie										
Robin	X					X				
Horned Owl										
Jap. Quail		X				X				
Rabbit	X					X				
Guinea Pig	X					X				
Golden Hamster	X					X				
Mouse (Ajax)	X					X				
Field Mouse						X				
Rat				X			X			
Cow		X					X			
Sheep	X					X				
Horse		X				X				
Pig	X					X				
Cat	X					X				
Dog										
Man (mature)										
Man (infant)	X					X				

Table I-33	ENZYME: Acid Phosphatase (Gomori)					ENZYME: Acid Phosphatase (Barka)				
TISSUE CELLS:	Brain, Perithelial microglia									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike			X					X		
Northern Pike			X							X
Newt	X					X				
Salamander	X					X				
Grass Frog			X				X			
Tree Frog	X						X			
Turtle	X						X			
Chamelion	X					X				
Chicken			X				X			
Magpie										
Robin				X						X
Horned Owl										
Jap. Quail		X						X		
Rabbit		X					X			
Guinea Pig			X					X		
Golden Hamster					X					X
Mouse (Ajax)			X					X		
Field Mouse										
Rat					X					X
Cow				X					X	
Sheep	X							X		
Horse	X						X			
Pig			X					X		
Cat	X					X				
Dog										
Man (mature)										
Man (infant)			X					X		

Table I-33	ENZYME: Glucuronidase					ENZYME: glucosaminidase				
TISSUE CELLS:	Brain, Perithelial microglia									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X					X				
Northern Pike					X					X
Newt	X					X				
Salamander	X					X				
Grass Frog	X					X				
Tree Frog	X					X				
Turtle		X								X
Chamelion	X					X				
Chicken	X							X		
Magpie										
Robin		X					X			
Horned Owl										
Jap. Quail		X								X
Rabbit	X					X				
Guinea Pig				X						X
Golden Hamster			X			X				
Mouse (Ajax)					X				X	
Field Mouse										
Rat				X						X
Cow		X								
Sheep		X					X			
Horse	X								X	
Pig			X				X			
Cat				X					X	
Dog										
Man (mature)										
Man (infant)		X				X				

Table I-33	ENZYME: N - Acetate Esterase					ENZYME: N - AS - Acetate Esterase				
TISSUE CELLS:	Brain, Perithelial microglia									
	ENZYME STAIN					ENZYME STAIN				
ANIMAL:	0	I+	2+	3+	4+	0	I+	2+	3+	4+
Walleye Pike	X									
Northern Pike	X								X	
Newt	X					X				
Salamander	X					X				
Grass Frog	X						X			
Tree Frog		X					X			
Turtle		X				X				
Chamelion	X					X				
Chicken				X		X				
Magpie										
Robin		X						X		
Horned Owl										
Jap. Quail	X					X				
Rabbit	X							X		
Guinea Pig	X					X				
Golden Hamster	X									X
Mouse (Ajax)				X						X
Field Mouse										
Rat					X					X
Cow				X			X			
Sheep			X				X			
Horse				X						X
Pig				X				X		
Cat			X					X		
Dog										
Man (mature)										
Man (infant)					X					X

TABLE II.

Survey of Acid Hydrolase Content of Different Tissues of Individual Vertebrate Species.

Table II-1 to Table II-28 inclusive.

Table II-1

ANIMAL FISH II (Stizostedion Vitreum) TISSUES	E N Z Y M E					
	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N SM 3+	N S 4+	N SM 3+	N S ±	d 3+	d 4+
Mid portion	F SM 1+	N S 3+	N S 1+	- - -	d 1+	Gd 1+
Distal portion	F SM 1+	F S 1+	- - -	- - -	d 1+	Gd 1+
Distal tubule						
Loop of Henle						
Collecting tubule						
Principal cells	F S 1+	N S 1+	- - -	- - -	- - -	d 2+
Intercalated cells						
Glomerulus	- - -	N S 3+	- - -	- - -	- - -	d 2+
Visc. epith.	- - -	- - -	- - -	- - -	- - -	- - -
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	F S 1+	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	F 2+	N 3+	N 3+	F 2+	N 3+	N 3+
Peripheral	F 2+	N 3+	N 3+	F 2+	N 2+	N 3+
Parenchymal cells						
Pericanalicular	F S 3+	N M 3+	N S 3+	F S 2+	N S 3+	F S 4+
Perinuclear	- - -	- - -	- - -	- - -	F SG 1+	N SG 3+
Kupffer cells	F M 3+	F M 4+	F M 3+	F M 3+	F SG 2+	F S 3+
Bileduct epithelium	- - -	F S 1+	- - -	- - -	- - -	F SG 1+
PANCREAS						
Islet cells	- - -	d 1+	- - -	F Sd 2+	F Sd 1+	N G ±
Acinar cells	F S 2+	d 1+	- - -	- - -	Gd ±	N G 3+
Duct epithelium	F S ±	d ±	F S ±	F S -	- - -	N G 2+

Table II-1

ANIMAL FISH II (Stizostedion Vitreum) TISSUE	ENZYME APase Gomori No.Si.St.	 APase Barka No.Si.St.	 Glucuron-idase No.Si.St.	 Glucos-aminidase No.Si.St.	 N-Acetate Esterase No.Si.St.	 N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N S 3+	N SL 4+	N SL 4+	N SL 4+	N SL 3+	N SL 3+
Cords of Billroth	F S ±	F S 1+	- - -	F SM 1+	- - -	N G ±
White pulp						
Macrophages	N S 3+	N SL 4+	N SL 4+	N SL 4+	N SL 3+	N SL 3+
Lymphoid cells	F S ±	F S 2+	- - -	F S 1+	- - -	N G ±
DUODENUM						
Villus, epithelium						
Apex	F S 1+	N S 3+	F Sd ±	N SM 1+	N SLd 3+	N Gd 4+
Middle	F S 1+	N S 3+	F Sd ±	d ±	N SLd 3+	N G 3+
Crypt	F S 1+	N S 3+	F Sd ±	- - -	d 2+	N G 3+
Lamina propria macroph.	N SM 4+	N SL 4+	- - -	F S ±	N S-L 4+	N SL 4+
BRAIN						
Neurones Cortical	- - -	N Sd 2+	- - -	- - -	d ±	
Purkinje	- - -	d 1+	- - -	- - -	- - -	
Glial cells	- - -	- - -	- - -	- - -	- - -	
Endothelial cells	- - -	- - -	- - -	- - -	- - -	
Perithelial microglia cells	F S 2+	F S 2+	- - -	- - -	- - -	

Table II-2

ANIMAL FISH I (Esox Lucius)	E N Z Y M E					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N M 4+	N SM 4+	F SM 3+	N SM 3+	d 3+	SG 3+
Mid portion	N SM 3+	N S 2+	F SM 2+	N SM 2+	d 2+	SG 2+
Distal portion	N SM 3+	N S 3+	F SM 2+	N SM 2+	d 2+	SG 2+
Distal tubule						
Loop of Henle						
Collecting tubule						
Principal cells	F S 1+	F S 1+	- - -	- - -	d 1+	SG 1+
Intercalated cells						
Glomerulus	F S 1+	N S 2+	- - -	- - -	d 1+	SG 1+
Visc. epith.	- - -	- - -	- - -	- - -	- - -	- - -
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 4+	N 4+	N 4+	N 3+	d 3+	N Gd 3+
Peripheral	N 4+	N 4+	N 4+	N 3+	d 3+	N Gd 3+
Parenchymal cells						
Pericanalicular	N SL 4+	N M 4+	N SM 4+	N SM 3+	Gd 3+	N G 3+
Perinuclear	- - -	- - -	- - -	- - -	Gd 3+	N G 3+
Kupffer cells	F L 4+	F L 4+	F SM 4+	F L 4+	F G 3+	Gd 2+
Bileduct epithelium	N S 1+	N S 3+	F S 1+	- - -	- - -	F G 1+
PANCREAS						
Islet cells	N Sd 3+	N S 4+	- - -	N Sd 3+	N Gd 3+	N Sd 4+
Acinar cells	N S 1+	N S 2+	- - -	- - -	F S ±	N Sd 2+
Duct epithelium	F S ±	N S 2+	- - -	- - -	- - -	- - -

Table II-2

ANIMAL FISH I (Esox Lucius)	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N S 2+	N SL 4+	F SM 3+	F SM 3+	F S 3+	N MG 4+
Cords of Billroth	F S ±	F S 2+	- - -	- - -	F G ±	N G 1+
White pulp						
Macrophages	N S 2+	N SL 4+	N SM 2+	F SM 3+	F S 3+	N G 4+
Lymphoid cells	F S ±	F S 2+	- - -	- - -	- - -	N G 1+
DUODENUM						
Villus, epithelium						
Apex	N S 4+	N S 4+	- - -	N SM 3+	d 4+	N G 3+
Middle	N S 4+	N S 4+	- - -	N S 3+	d 2+	N G 3+
Crypt	N S 2+	N S 4+	- - -	N S 2+	d 2+	N G 3+
Lamina propria macroph.	N S 3+	N SM 4+	F M 2+	F SM 4+	d 1+	F G 3+
BRAIN						
Neurones Cortical	N S 4+	N Sd 3+	- - -	- - -	- - -	N G 1+
Purkinje	d ±	d 2+	- - -	- - -	- - -	- - -
Glial cells	- - -	N S 4+	- - -	- - -	d 1+	N G 2+
Endothelial cells	F S ±	F S 1+	- - -	- - -	- - -	N G ±
Perithelial microglia cells	N SM 2+	N S 4+	N SM 4+	N SM 4+	- - -	N SG 3+

Table II-3

ANIMAL NEWT		ENZYME					
TISSUES		APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion						
	Mid portion						
	Distal portion						
	Distal tubule						
	Loop of Henle						
	Collecting tubule						
	Principal cells						
	Intercalated cells						
	Glomerulus						
	Visc. epith.						
	Parietal epith.						
	Endothelium						
LIVER	Lobule						
	Central			N 3+			
	Peripheral			N 3+			
	Parenchymal cells						
	Pericanalicular			N S 3+			
	Perinuclear			F S 1+			
	Kupffer cells			F S 3+			
	Bileduct epithelium			- - -			
PANCREAS							
	Islet cells						
	Acinar cells						
	Duct epithelium						

Table II-3

ANIMAL NEWT	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	F SM 4+	F S 3+	d ±	d ±	- - -	- - -
Cords of Billroth	- - -	F S 2+	- - -	- - -	d ±	- - -
White pulp						
Macrophages	F SM 4+	N SG 4+	F S 2+	F S 3+	- - -	F SG 1+
Lymphoid cells	- - -	F Sd 3+	- - -	- - -	- - -	F G ±
DUODENUM						
Villus, epithelium						
Apex	N SG 3+	N S 3+	N S 2+	N S 3+		N G 3+
Middle	N S 3+	N S 3+	N S 2+	N S 3+		N G 3+
Crypt	N S 2+	N S 3+	N S ±	N S 1+		N G 3+
Lamina propria macroph.	F S 3+	F Sd 3+	F S 2+	F S 4+		- - -
BRAIN						
Neurones Cortical	- - -	- - -	N SM 3+	N SM 4+	- - -	N G 1+
Purkinje	- - -	- - -	- - -	- - -	- - -	- - -
Glial cells	- - -	- - -	- - -	- - -	- - -	- - -
Endothelial cells	- - -	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	- - -	- - -	- - -	- - -	- - -	- - -

Table II-4

ANIMAL	SALAMANDER	E N Z Y M E					
TISSUES		APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion						
	Mid portion						
	Distal portion						
	Distal tubule						
	Loop of Henle						
	Collecting tubule						
	Principal cells						
	Intercalated cells						
	Glomerulus						
	Visc. epith.						
	Parietal epith.						
	Endothelium						
LIVER	Lobule						
	Central		N 3+	F 1+	F 3+	G 1+	- - -
	Peripheral		N 3+	F 1+	F 3+	G 1+	- - -
	Parenchymal cells						
	Pericanalicular		N SM 3+	F SM 4+	F SM 4+	Gd 1+	F S 1+
	Perinuclear		- - -	- - -	- - -	- - -	- - -
	Kupffer cells		N Md 4+	F Sd 4+	N Md 4+	Gd 2+	- - -
	Bileduct epithelium		- - -	- - -	- - -	- - -	- - -
PANCREAS							
	Islet cells	F S 1+	F S 1+	- - -	F S 2+	d ±	N G 1+
	Acinar cells	F S 1+	F S 2+	- - -	F S 2+	d 1+	N G 2+
	Duct epithelium	- - -	F S 1+	- - -	- - -	d ±	N G 1+

Table II-4

ANIMAL SALAMANDER	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	F SM 3+	N S 4+	d 2+	N SM 4+	d ±	F G 1+
Cords of Billroth	- - -	F Sd 2+	- - -	- - -	- - -	- - -
White pulp						
Macrophages	F SM 3+	N SM 4+	d ±	N SM 4+	- - -	F G ±
Lymphoid cells	- - -	F Sd 2+	- - -	- - -	- - -	- - -
DUODENUM						
Villus,epithelium						
Apex	F S 1+	N Sd 3+	- - -	N S 3+	d 4+	N G 3+
Middle	F S 1+	N Sd 3+	- - -	N S 3+	d 3+	N G 3+
Crypt	F S 1+	N Sd 3+	- - -	N S 3+	d 3+	N G 3+
Lamina propria macroph.	- - -	N S 4+	- - -	N SM 4+	F Gd 1+	F G ±
BRAIN						
Neurones Cortical	N SM 4+	N SM 4+	- - -	N SM 4+	- - -	F S 2+
Purkinje	- - -	- - -	- - -	- - -	- - -	- - -
Glial cells	- - -	- - -	- - -	- - -	- - -	- - -
Endothelial cells	- - -	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	- - -	- - -	- - -	- - -	- - -	- - -

Table II-5

ANIMAL FROG (RANA PIPIENS)		ENZYME					
TISSUES		APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion	N SL 4+	N S 4+	N S 2+	N SM 4+	d 4+	N M 4+
	Mid portion						
	Distal portion					d 1+	
	Distal tubule	N S 2+	F S 1+	- - -	- - -	d 1+	SG 1+
	Loop of Henle	F S 1+	F S 1+	- - -	- - -		G 1+
	Collecting tubule						
	Principal cells	F S 1+	F S 1+	- - -	- - -	- - -	G 1+
	Intercalated cells						
	Glomerulus						
	Visc. epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	N 4+	N 3+	N 2+	N 1+	d 3+	N 3+
	Peripheral	N 4+	N 3+	N 2+	N 1+	d 3+	N 3+
	Parenchymal cells						
	Pericanalicular	N S 4+	N S 3+	N S 2+	N S 1+	Gd 4+	N SG 3+
	Perinuclear	- - -	F S 1+	- - -	- - -	Gd 1+	N SG 2+
	Kupffer cells	F L 4+	F S 3+	N SL 4+	F SM 2+	N Sd 4+	N SG 4+
	Bileduct epithelium	- - -	N S 4+	F S 1+	N S 2+	N SG 4+	N SG 4+
PANCREAS							
	Islet cells	F S ±	N S 2+	- - -	- - -	N G 2+	N Gd 3+
	Acinar cells	F S ±	N S 2+	- - -	- - -	N SG 2+	N Gd 3+
	Duct epithelium	F s 2+	N S 1+	N S 4+	N S 4+	F Sd 2+	N G 4+

Table II-5

ANIMAL FROG (RANA PIPIENS)	E N Z Y M E					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N S 3+	N Sd 4+	N S 3+	F Sd ±	d 3+	N Sd 3+
Cords of Billroth	N S 1+	F Sd 2+	F S 1+	- - -	- - -	- - -
White pulp						
Macrophages	N S 3+	N Sd 4+	N SM 4+	F Sd ±	N SGd 2+	N G 3+
Lymphoid cells	N S 1+	F Sd 1+	- - -	- - -	d ±	F G 1+
DUODENUM						
Villus, epithelium						
Apex	F S 1+	N SMd 4+	F SMd 1+	N Sd 4+	d 4+	N G 4+
Middle	F S 1+	N SMd 4+	d 1+	N Sd 4+	d 4+	N G 4+
Crypt	F S 1+	N Sd 4+	d ±	N S 3+	d 4+	N G 2+
Lamina propria macroph.	N S 3+	F S 2+	N SM 3+	d 2+	d ±	F G 1+
BRAIN						
Neurones Cortical	N SM 4+	N S 3+	- - -	- - -	- - -	N G 1+
Purkinje		F S 1+	- - -	- - -	- - -	- - -
Glial cells	N S 3+	d ±	- - -	- - -	- - -	- - -
Endothelial cells	- - -	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	N S 2+	F S 1+	- - -	- - -	- - -	N G 1+

Table II-6

ANIMAL TOAD	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N M 4+	N M 4+	N S 2+	N M 3+	N Ld 4+	N Md 4+
Mid portion	N M 2+	N M 3+	N S 1+	N S 2+	N Gd 4+	N Md 3+
Distal portion		F S 2+	N S 1+	N S 1+	N Gd 1+	
Distal tubule	F SM 2+	- - -	- - -	- - -	Gd 1+	N G 2+
Loop of Henle	F M 1+	F S 1+	- - -	- - -	Gd 1+	N Gd 1+
Collecting tubule						
Principal cells	F M 1+	F S 1+	- - -	- - -	N Gd 2+	N M 4+
Intercalated cells						
Glomerulus	F M 1+	F S 1+	- - -	- - -	N G 1+	G 1+
Visc. epith.	F S 1+	F S 1+	- - -	- - -	d 1+	Gd 1+
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 3+	N 3+	N 2+	- - -	N 4+	N 4+
Peripheral	N 3+	N 3+	N 2+	- - -	N	N 4+
Parenchymal cells						
Pericanalicular	N M 3+	N S 4+	F SM 2+	- - -	N S 4+	N S 3+
Perinuclear	F S ±	N S 4+	- - -	- - -	N S 4+	N S 3+
Kupffer cells	F L 4+	N SM 4+	N S 3+	N S 2+	N S 4+	N SM 4+
Bileduct epithelium	N S 3+	N S 3+	- - -	- - -	N S 3+	N S 2+
PANCREAS						
Islet cells	N S 3+	N S 3+	- - -	d ±	- - -	N G 1+
Acinar cells	F S 2+	F S 1+	- - -	- - -	N SG 2+	N Gd 3+
Duct epithelium	F S 1+	N S 2+	- - -	- - -	d 1+	N G 1+

Table II-6

ANIMAL TOAD	E N Z Y M E					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages		F Sd 2+				
Cords of Billroth		F Sd 1+				
White pulp						
Macrophages		F Sd 2+				
Lymphoid cells		F Sd 1+				
DUODENUM						
Villus, epithelium						
Apex	N SM 4+	N SM 4+	N S ±	N S 3+	d 3+	N Sd 4+
Middle	N S 4+	N SM 4+	N S ±	N S 3+	d 3+	N Sd 4+
Crypt	N S 3+	N S 3+	N S ±	F S ±	d 4+	N Sd 3+
Lamina propria macroph.	N SL 4+	N SL 4+	N SM 3+	F S 4+	d 4+	N SL 4+
BRAIN						
Neurones Cortical	F S 1+	F Sd ±	- - -	- - -	N Gd 2+	N G 4+
Purkinje	- - -	d +	- - -	- - -	- - -	N G 2+
Glial cells	- - -	F S ±	- - -	- - -	- - -	F G 1+
Endothelial cells	- - -	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	- - -	F S ±	- - -	- - -	F Gd ±	F G 1+

Table II-7

ANIMAL TURTLE	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N M 3+	N M 4+	F S 1+	N SM 4+	Gd 4+	N G 2+
Mid portion	F S 1+	N M 3+	- - -	N SM 3+	Gd 4+	N G 2+
Distal portion	F S 1+	N M 2+	- - -	N SM 3+	Gd 4+	N G 2+
Distal tubule	- - -	F S 1+	d 1+	N SM 2+	SG 1+	N G 1+
Loop of Henle	- - -	F S 1+	- - -	F S 1+	SG 1+	- - -
Collecting tubule						
Principal cells	F S 2+	F S 2+	- - -	N S 2+	Gd 1+	F S ±
Intercalated cells						
Glomerulus	F S 2+	F S 3+	- - -	N S 2+	Gd 1+	F S ±
Visc. epith.	- - -	F Sd 1+	- - -	F S 1+	- - -	- - -
Parietal epith.	- - -	F Sd 1+	- - -	F S 1+	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 2+	N 2+	N 3+	N 2+	G 2+	- - -
Peripheral	N 2+	N 2+	N 3+	N 2+	G 2+	- - -
Parenchymal cells						
Pericanalicular	N S 2+	N Gd 2+	N S ±	N S 3+	Gd 2+	- - -
Perinuclear	- - -	- - -	- - -	- - -	Gd 2+	- - -
Kupffer cells	- - -	Gd 2+	N S 4+	Gd 4+	Gd 3+	- - -
Bileduct epithelium	- - -	F S 3+	- - -	F S 2+	F S 2+	- - -
PANCREAS						
Islet cells	F S 1+	N S 2+	F S 1+	N SG 3+	d ±	- - -
Acinar cells	F S ±	F Sd 1+	- - -	- - -	d 3+	N G 2+
Duct epithelium	- - -	N S 1+	F S ±	N S 1+	F S ±	- - -

Table II-7

ANIMAL TURTLE	E N Z Y M E					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	F S 3+	F Sd 3+	d 1+	F S 3+	d 4+	N G 1+
Cords of Billroth	- - -	d ±	- - -	- - -	- - -	- - -
White pulp						
Macrophages	F S 3+	F Sd 3+	F S 2+	N S 4+	d 1+	F G 1+
Lymphoid cells	- - -	d 1+	- - -	F S ±	- - -	- - -
DUODENUM						
Villus,epithelium						
Apex						
Middle						
Crypt						
Lamina propria macroph.						
BRAIN						
Neurones Cortical	N S 3+	N S 4+	F Sd 1+	N S 4+	- - -	- - -
Purkinje	- - -	- - -	- - -	- - -	- - -	- - -
Glial cells	- - -	N S 3+	- - -	F S 2+	- - -	- - -
Endothelial cells	- - -	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	- - -	F S ±	F Sd 1+	N S 4+	d 1+	- - -

Table II-8

ANIMAL CHAMELION	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	F SM 2+	N S 3+	- - -	F S ±	G 3+	G 3+
Mid portion	F SM 2+	N S 1+	- - -	- - -	G 3+	G 1+
Distal portion			- - -	- - -	G 3+	
Distal tubule	- - -	- - -	- - -	- - -	G 3+	- - -
Loop of Henle	- - -		- - -	- - -	- - -	- - -
Collecting tubule						
Principal cells	- - -	F S 1+	- - -	- - -	- - -	- - -
Intercalated cells						
Glomerulus	- - -	F S 1+	- - -	- - -	- - -	- - -
Visc. epith.	- - -	- - -	- - -	- - -	- - -	- - -
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	F 2+	N 2+	- - -	- - -	N 3+	N 3+
Peripheral	F 2+	N 2+	- - -	- - -	N 3+	N 3+
Parenchymal cells						
Pericanalicular	F S 2+	N M 2+	- - -	- - -	N M 3+	N M 3+
Perinuclear	F S ±	F S 1+	- - -	- - -	N M 3+	N S 3+
Kupffer cells	F SM 2+	F S 3+	F Sd ±	- - -	F S 1+	F S 1+
Bileduct epithelium	F S 1+	- - -	- - -	- - -	F S 2+	N S 2+
PANCREAS						
Islet cells	- - -	- - -	- - -	F S ±	- - -	N G 1+
Acinar cells	F S 1+	F S ±	- - -	F S ±	d 1+	N G 2+
Duct epithelium	- - -	- - -	- - -	- - -	- - -	N G 1+

Table II-8

ANIMAL CHAMELION	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	- - -	F Sd 1+	- - -	F S 1+	N Gd 3+	F G 1+
Cords of Billroth	- - -	F Sd ±	- - -	F S 1+	F Gd 1+	- - -
White pulp						
Macrophages	- - -	F Sd 1+	- - -	F S 1+	N Gd 2+	F G 1+
Lymphoid cells	- - -	F Sd ±	- - -	F S 1+	F Sd 1+	- - -
DUODENUM						
Villus, epithelium						
Apex	N SM 4+	N SM 4+	- - -	F Sd ±	N Gd 4+	N Gd 3+
Middle	N SM 4+	N SM 4+	- - -	F Sd ±	N Gd 4+	N Gd 3+
Crypt	N SM 4+	N S 2+	- - -	F Sd ±	N Gd 3+	N Gd 3+
Lamina propria macroph.	F S 1+	F Sd 4+	d ±	- - -	F G 1+	- - -
BRAIN						
Neurones Cortical	- - -	- - -	- - -	- - -	- - -	- - -
Purkinje	- - -	- - -	- - -	- - -	- - -	- - -
Glial cells	- - -	- - -	- - -	- - -	- - -	- - -
Endothelial cells	- - -	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	- - -	- - -	- - -	- - -	- - -	- - -

Table II-9

ANIMAL CHICKEN		ENZYME					
TISSUES		APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion	N S 3+	N SM 4+	F Sd 2+	F S 3+	N Gd 4+	N G 2
	Mid portion	N S 3+	N S 3+	F Sd 1+	F S 2+	N Gd 4+	N G 1
	Distal portion	N S 1+	N S 2+	F Sd 1+	F S 2+	N Gd 4+	N G 1
	Distal tubule	F S 1+	N S 1+	F Sd ±	F S 1+	- - -	- - -
	Loop of Henle						
	Collecting tubule						
	Principal cells	F S 1+	F S 2+	F Sd ±	F S ±	- - -	- - -
	Intercalated cells	F S 3+	F S 2+	F Sd ±	F S ±	- - -	- - -
	Glomerulus						
	Visc. epith.	F S ±1	F Sd 1+	- - -	- - -	N Gd 3+	N G 2+
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	F 2+	F 2+	1+	F 1	N G 3+	N G 2+
	Peripheral	F 2+	F 2+	1+	F 1	N G 4+	N G 3+
	Parenchymal cells						
	Pericanalicular	N S 2+	N S 2+	d ±	- - -	N Gd 3+	N G 3+
	Perinuclear	- - -	- - -	- - -	- - -	N Gd 3+	- - -
	Kupffer cells	N SL 4+	F SM 4+	F SMd 1+	F SL 3+	N Gd 3+	N G 2+
	Bileduct epithelium	F S 1+	F S 1+	- - -	- - -	- - -	- - -
PANCREAS							
	Islet cells	F S 1+	N S 3+	- - -	d ±	- - -	N G 1+
	Acinar cells	N SM 4+	N S 4+	F Sd ±	- - -	N Gd 3+	F G ±
	Duct epithelium	N S 3+	N S 4+	N Sd 2+	F S 3+	N d 4	N SG 2+

Table II-9

ANIMAL CHICKEN	E N Z Y M E					
TISSUE	APase Comori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N S 4+	N SL 4+	F SLd 3+	F SM 4+	F G 1+	- - -
Cords of Billroth	F L 1+	N S 1+	- - -	F S 1+	- - -	- - -
White pulp						
Macrophages	F SL 3+	N SL 4+	F Sd ±	F SM 3+	- - -	- - -
Lymphoid cells	N S 2+	N S 2+	- - -	- - -	- - -	- - -
DUODENUM						
Villus, epithelium						
Apex	F SM 3+	N S 3+	d ±	N S 2+	N Gd 4	N Gd 4
Middle	N S 3+	N S 3+	d ±	N S 2+	N Gd 4	N Gd 4
Crypt	N S 2+	N S 2+	d ±	N S 2+	N Gd 4	N Gd 4
Lamina propria macroph.	N SL 4+	N SL 4+	F SM ±	F SL 4+	F SG 1+	- - -
BRAIN						
Neurones Cortical	N S 2+	N S 2+	d 1+	d ±	N Gd 2+	- - -
Purkinje	F S 1+	N S 2+	d 1+	d ±	N Gd 2+	- - -
Glial cells	F S ±	F S 2+	- - -	- - -	d 1+	- - -
Endothelial cells	F S ±	F S ±	- - -	- - -	- - -	- - -
Perithelial microglia cells	N S 2+	F S 1+	- - -	F S 2+	N Gd 3+	- - -

Table II-10

ANIMAL MAGPIE		ENZYME					
TISSUES		APase Comori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion	F SM 3+	F SM 4+	F S 4+	F M 3+	d 3+	Gd 3+
	Mid portion						
	Distal portion						
	Distal tubule	F S ±	F S ±	- - -	- - -	- - -	- - -
	Loop of Henle						
	Collecting tubule						
	Principal cells						
	Intercalated cells	- - -					
	Glomerulus	F S ±	F S 1+	F S 1+	- - -	- - -	- - -
	Visc. epith.	F S ±	d ±	F S 1+	- - -	- - -	F G ±
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	N 4+	N 3+	N 3+	F 1+	d 4+	N Gd 3+
	Peripheral	N 4+	N 4+	N 3+	F 1+	d 4+	N Gd 3+
	Parenchymal cells						
	Pericanalicular	N S 4+	N S 4+	N S 3+	N S 1+	Gd 4+	N SG 3+
	Perinuclear	- - -	- - -	- - -	- - -	Gd 4+	N SG 3+
	Kupffer cells	N SL 4+	N M 4+	F M 4+	F SM 3+	Gd 4+	N SG 3+
	Bileduct epithelium	F S 1+	N S 3+	F S 3+	- - -	Gd 1+	F SG 1+
PANCREAS							
	Islet cells	N S 1+	N Sd 1+	d ±	- - -	d 1+	F G 1+
	Acinar cells	F S ±	F Sd ±	- - -	- - -	d 1+	F G 1+
	Duct epithelium	F S 1+	- - -	- - -	- - -	d 1+	F G 1+

Table II-10

ANIMAL MAGPIE	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N SL 4+	N SM 3+	F S 3+	F S 1+	F S 1+	F G 1+
Cords of Billroth	F S 1+	F S ±	- - -	- - -	- - -	- - -
White pulp						
Macrophages	N SL 4+	N SM 3+	F S 3+	F S 1+	F S 1+	F SG 1+
Lymphoid cells	- - -	F S ±	- - -	- - -	- - -	- - -
DUODENUM						
Villus,epithelium						
Apex	N S 3+	F Sd 2+	F Sd 1+	- - -	N Gd 4+	N G 3+
Middle	N S 3+	F Sd 2+	F Sd 1+	- - -	N Gd 4+	N G 3+
Crypt	N S 3+	F Sd 2+	F Sd 1+	- - -	N Gd 4+	N G 3+
Lamina propria macroph.	F SM 3+	N SM 4+	F SM 4+	F S 2+	N Gd 4+	F G ±
BRAIN						
Neurones Cortical						
Purkinje						
Glial cells						
Endothelial cells						
Perithelial microglia cells						

Table II-11

ANIMAL ROBIN	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N SM 4+	N S 4+	F S 4+	F S 4+	d 4+	Gd 2+
Mid portion	F S 2+	N S 3+	d 1+	F S 1+	d 3+	Gd 2+
Distal portion	F S 1+	N S 2+	d 1+	F S 1+	d 3+	Gd 2+
Distal tubule	- - -	d 1+		F S 1+	d 2+	Gd 1+
Loop of Henle	F S 1+	F Sd 1+	- - -	- - -	- - -	- - -
Collecting tubule						
Principal cells	F S 1+	F S ±	- - -	F S ±	F SG 2+	Gd 1+
Intercalated cells						
Glomerulus	F S 2+	F S 2+	- - -	F S 4+	Gd 3+	Gd 2+
Visc. epith.	F S 1+	- - -	- - -	- - -	- - -	F S 3+
Parietal epith.	- - -	- - -	- - -	- - -	- - -	F G ±
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	F 2+	N 2+	d 1+	F 1+	d 3+	G 1+
Peripheral	F 2+	N 2+	d 1+	F 2+	d 3+	G 1+
Parenchymal cells						
Pericanalicular	N S 2+	F S ±	Gd 1+	F S 1+	d 3+	N G 1+
Perinuclear	- - -	- - -	- - -	- - -	d 3+	N G 1+
Kupffer cells	N S 4+	N Sd 4+	F M 4+	F SM 4+	Gd ±	F S 4+
Bileduct epithelium	F S 1+	- - -	- - -	- - -	- - -	- - -
PANCREAS						
Islet cells	d 1+	d 1+	d ±	N S 3+	d 1+	N G 1+
Acinar cells	N S 3+	N S 4+	- - -	- - -	d 2+	N G 2+
Duct epithelium	- - -	F S 1+	d ±	- - -	d 1+	N G 1+

Table II-11

ANIMAL ROBIN	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	F SM 4+	N S 4+	N S 3+	N SM 3+	d ±	F G 1+
Cords of Billroth	F S 2+	F Sd 2+	F Sd ±	- - -	- - -	- - -
White pulp						
Macrophages	F SM 4+	N SM 4+	F Sd ±	F S 1+	- - -	F G ±
Lymphoid cells	- - -	F Sd 2+	- - -	N SM 3+	- - -	- - -
DUODENUM						
Villus, epithelium						
Apex	N SM 3+	N Sd 4+	F Sd ±	F Sd 1+	d 4+	N G 2+
Middle	N S 3+	N Sd 4+	F Sd ±	F Sd 1+	d 3+	N G 2+
Crypt	N S 2+	N Sd 4+	F Sd ±	F Sd 1+	d 3+	N G 2+
Lamina propria macroph.	F S ±	N SMd 4+	F Sd 1+	F SM 3+	F Gd 1+	F G 1+
BRAIN						
Neurones Cortical	N S 3+	F Sd 2+	- - -	F Sd 1+	d 1+	N G 1+
Purkinje	- - -	- - -	- - -	- - -	d 1+	N G 3+
Glial cells	F S ±	- - -	- - -	- - -	- - -	- - -
Endothelial cells	F S ±	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	F S 3+	F SM 4+	d ±	F Sd 1+	d 1+	N G 2+

Table II-12

ANIMAL HORNED OWL		ENZYME					
TISSUES		APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion	N M 3+	N S 3+	- - -	N SM 4+	d 4+	Gd 3+
	Mid portion						
	Distal portion						
	Distal tubule	- - -	F S ±	- - -	- - -	d 1+	- - -
	Loop of Henle						
	Collecting tubule						
	Principal cells		- - -	- - -	- - -	- - -	- - -
	Intercalated cells	- - -	N S 2+	- - -	F S 1+	- - -	- - -
	Glomerulus	F SM 2+	- - -	- - -	F S 1+	Gd 1+	F S 1+
	Visc. epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	N 3+	N 3+	- - -	d ±	N 4+	N 2+
	Peripheral	N 3+	N 3+	- - -	d ±	N 4+	N 2+
	Parenchymal cells						
	Pericanalicular	N S 3+	N S 3+	- - -	N S ±	N M 4+	N G 2+
	Perinuclear	- - -	- - -	- - -	- - -	N S 3+	N G 2+
	Kupffer cells	F M 4+	N S 4+	- - -	F SM 4+	F Sd 1+	N S 4+
	Bileduct epithelium	F S 1+	- - -	- - -	- - -	F Sd 1+	d ±
PANCREAS							
	Islet cells	F S 1+	d 1+	- - -	F Sd -	- - -	F G 1+
	Acinar cells	F S ±	d ±	- - -	- - -	d 2+	F G 1+
	Duct epithelium	- - -	- - -	- - -	- - -	- - -	F G 1+

Table II-12

ANIMAL HORNED OWL	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N SM 3+	d 2+	- - -	F SM 4+	- - -	- - -
Cords of Billroth	F S 1+	d 1+	- - -	- - -	- - -	- - -
White pulp						
Macrophages	N SM 3+	d 3+	- - -	F S ±	F Sd ±	- - -
Lymphoid cells	F S ±	- - -	- - -	- - -	- - -	F G ±
DUODENUM						
Villus, epithelium						
Apex	N S 2+	d 1+	- - -	F S 1+	d 4+	N G 2+
Middle	N S 2+	d 1+	- - -	F S 1+	d 4+	N G 2+
Crypt	N S 2+	d 1+	- - -	F S 1+	d 4+	N G 1+
Lamina propria macroph.	N S 2+	- - -	- - -	F S 2+	F Gd 2+	F G ±
BRAIN						
Neurones Cortical						
Purkinje						
Glial cells						
Endothelial cells						
Perithelial microglia cells						

Table II-13

ANIMAL JAP. QUAIL	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N M 4+	N M 4+	N M 4+	N SM 4+	d 4+	Gd 3+
Mid portion						
Distal portion		F S 2+		- - -		
Distal tubule	F S ±	- - -	- - -	- - -	- - -	F G ±
Loop of Henle						
Collecting tubule						
Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
Intercalated cells	F M 2+	F S 2+	F S 1+	F S 1+	- - -	- - -
Glomerulus	F S 1+	F S 1+	F S 2+	F S 1+	- - -	F S 1+
Visc. epith.	- - -	- - -	- - -	F S ±	- - -	F G 1+
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 4+	F 2+	F 2+	F 1+	d 4+	N G 3+
Peripheral	N 4+	F 3+	F 2+	F 1+	d 4+	N G 3+
Parenchymal cells						
Pericanalicular	N S 4+	F S 2+	F S 2+	F S 1+	Gd 4+	N G 3+
Perinuclear	d 1+	- - -	d 1+	- - -	Gd 4+	N G 3+
Kupffer cells	F M 4+	N M 4+	F S 3+	F S 4+	F Gd 1+	N G 2+
Bileduct epithelium	- - -	- - -	F Sd 1+	- - -	- - -	F G 1+
PANCREAS						
Islet cells	F S 1+	N S 2+	- - -	F S 1+	N G 1+	N G 2+
Acinar cells	N S 3+	N S 3+	- - -	F S ±	N Gd 2+	N G 2+
Duct epithelium	F S 1+	F S 1+	- - -	- - -	- - -	- - -

Table II-14

ANIMAL JAP. QUAIL	E N Z Y M E					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages						
Cords of Billroth						
White pulp						
Macrophages						
Lymphoid cells						
DUODENUM						
Villus, epithelium						
Apex	N S 4+	N Sd 3+	N S 4+	N Sd 4+	d 4+	N G 4+
Middle	N S 4+	N Sd 3+	N S 4+	N Sd 4+	d 4+	N G 4+
Crypt	N S 4+	N Sd 2+	d 1+	F Sd 1+	d 4+	N G 4+
Lamina propria macroph.	N SM 4+	N S 4+	F S 3+	N SM 4+	- - -	F G ±
BRAIN						
Neurones Cortical	N S 2+	F S 2+	- - -	- - -	- - -	- - -
Purkinje	F S ±	F S 1+	- - -	- - -	- - -	- - -
Glial cells	F S ±	F S 2+	- - -	- - -	- - -	F G ±
Endothelial cells	- - -	- - -	- - -	- - -	d 1+	- - -
Perithelial microglia cells	N S +	d 2+	F SM 1+	N S 4+	- - -	- - -

Table II-15

ANIMAL RABBIT	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N M 4+	N S 3+	- - -	- - -	d 4+	d 4+
Mid portion						
Distal portion					d 3+	d 3+
Distal tubule	F S ±	F S ±	- - -	- - -	- -	- - -
Loop of Henle	- - -	- - -	- - -	- - -	- - -	- - -
Collecting tubule						
Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
Intercalated cells	N M 3+	N S 3+	F S ±	- - -	F S 2+	F S 2+
Glomerulus	- - -	- - -	- - -	- - -	F S 1+	F S 1+
Visc. epith.	- - -	F S 1+	- - -	- - -	- - -	- - -
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 2+	N 2+	N 3+	- - -	N 3+	N 3+
Peripheral	N 3+	N 3+	N 4+	- - -	N 4+	N 3+
Parenchymal cells						
Pericanalicular	N S 3+	N S 3+	N S 4+	- - -	N SG 3+	N SG 4+
Perinuclear	F S 1+	N S 1+	F S 1+	- - -	N SG 3+	N SG 3+
Kupffer cells	F L 4+	F L 4+	F L 4+	F S 2+	N SG 4+	N SG 4+
Bileduct epithelium	N S 2+	F S +	- - -	- - -	N SG 1+	N SG 3+
PANCREAS						
Islet cells	F Sd 1+	F d 1+	- - -	F d ±	- - -	F G 1+
Acinar cells	F S 1+	d ±	- - -	- - -	d 1+	N G 1+
Duct epithelium	- - -	- - -	- - -	- - -	d 2+	N G 3+

Table II-15

ANIMAL RABBIT	ENZYME																	
	APase Gomori			APase Barka			Glucuron-idase			Glucos-aminidase			N-Acetate Esterase			N-AS Acet Esterase		
TISSUE	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.
SPLEEN																		
Red pulp																		
Macrophages	N	SL	4+	N	S-L	4+	F	M	4+	-	-	-	F	d	2+	N	G	2+
Cords of Billroth	F	S	±	-	-	-	F	d	±	F	d	±	F	d	±	N	G	1+
White pulp																		
Macrophages	N	M	4+	-	-	-	F	d	1+	-	-	-	F	d	±	N	G	2+
Lymphoid cells	F	S	±	-	-	-	F	S	±	-	-	-	F	d	±	N	G	±
DUODENUM																		
Villus, epithelium																		
Apex	N	S	3+	F	Sd	1+	F	Sd	±	-	-	-	N	d	4+	N	G	3+
Middle	N	S	3+	F	Sd	1+	F	Sd	±	-	-	-		d	4+	N	G	3+
Crypt	N	S	3+	F	Sd	1+	F	Sd	±	-	-	-		d	3+	N	G	3+
Lamina propria macroph.	F	SM	3+	F	Sd	1+	F	Sd	2+	F	Sd	±		d	±	N	G	2+
BRAIN																		
Neurones Cortical	N	S	4+	N	S	2+	-	-	-	F	Sd	±	F	Sd	±	N	G	±
Purkinje	F	S	2+	F	Sd	1+	-	-	-	F	Sd	±	-	-	-	-	-	-
Glial cells	F	S	±	-	-	-	-	-	-	F	S	4+	-	-	-	-	-	-
Endothelial cells	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Perithelial microglia cells	F	S	±	F	S	1+	-	-	-	-	-	-	-	-	-	F	G	2+

Table II-16

ANIMAL GUINEA PIG	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N M 4+	N S 4+	- - -	N M 4+	Gd 4+	d 4+
Mid portion	N M 3+					
Distal portion	N S 3+	N S 3+	N S 2+	F S 2+	Gd 3+	
Distal tubule	- - -	- - -	- - -	- - -	- - -	- - -
Loop of Henle	- - -	- - -	- - -	F S 1+	F S 1+	F S 1+
Collecting tubule						
Principal cells	- - -	- - -	F S 1+	- - -	- - -	- - -
Intercalated cells	N S 4+	F S 2+	F S 3+	F S 3+	Gd 1+	F S 1+
Glomerulus	F S 1+	- - -	N S 2+	F S 1+	F S 1+	- - -
Visc. epith.	F S +	d ±	F S ±	F S 2+	- - -	- - -
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 3+	N 3+	N 2+	N 3+	d 3+	d 3+
Peripheral	N 4+	N 4+	N 3+	N 4+	d 3+	d 3+
Parenchymal cells						
Pericanalicular	N S 4+	N SM 3+	N S 4+	N S 4+	Gd 3+	Gd 3+
Perinuclear	- - -	F S 2+	- - -	- - -	Gd 3+	Gd 3+
Kupffer cells	F M 4+	N M 4+	F SL 3+	F M 4+	Gd 3+	Gd 4+
Bileduct epithelium	F S 1+	- - -	N M 4+	N S 4+	Gd 1+	Gd 3+
PANCREAS						
Islet cells	F S ±	d 1+	- - -	- - -	F G ±	F G ±
Acinar cells	F S ±	d 1+	- - -	- - -	Gd 2+	N G 2+
Duct epithelium	F S ±	d ±	F S ±	F S ±	- - -	N G 2+

Table II-16

ANIMAL GUINEA PIG	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N S-L 4+	N S-L 4+	N M 4+	N S 4+	N S 2+	N S 3+
Cords of Billroth	F S ±	d 1+	F S 1+	F S 1+	- - -	F S ±
White pulp						
Macrophages	N S 3+	F Sd 4+	N M 4+	N S-L 4+	d 2+	N G 3+
Lymphoid cells	F S ±	- - -	F S 1+	F S ±	- - -	F S ±
DUODENUM						
Villus, epithelium						
Apex	N S 3+	N Sd 3+	F Sd 1+	N S 2+	d 4+	N Gd 4+
Middle	N S 2+	N Sd 2+	F S 1+	N S 2+	d 4+	N Gd 3+
Crypt	N S 1+	N d 2+	F S 1+	F S ±	d 4+	N G 3+
Lamina propria macroph.	N S-L 4+	N M 4+	N M 4+	N M 4+	- - -	- - -
BRAIN						
Neurones Cortical	N S 3+	d 2+	- - -	N Sd 4+	- - -	- - -
Purkinje	F S 1+	d 2+	- - -	N S 2+	d -	- - -
Glial cells	N S 2+	F Sd 1+	- - -	N S 3+	- - -	F G ±
Endothelial cells	- - -	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	N S 2+	N Sd 2+	F S 3+	N S 4+	- - -	- - -

Table II-17

ANIMAL HAMSTER	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N M 4+	N S 4+	F S 1+	N S 1+	d 4+	Gd 4+
Mid portion				N S 2+	d 4+	
Distal portion	N S 3+	N S 3+	F S 1+	N S 1+	d 4+	Gd 2+
Distal tubule	F S ±	- - -	- - -	- - -	- - -	- - -
Loop of Henle	F S 1+	d ±	- - -	F S +	- - -	F S 1+
Collecting tubule						
Principal cells	N S 2+	d 1+	N S 4+	- - -	- - -	- - -
Intercalated cells	N L 4+	N S 3+	N S 4+	F S 3+	d 1+	N S 4+
Glomerulus	- - -	N d 1+	- - -	- - -	d 4+	F S 1+
Visc. epith.	- - -	F S +	F S +	- - -	- - -	F S 1+
Parietal epith.	- - -	- - -	- - -	- - -	- - -	F S 1+
Endothelium	- - -	d ±	- - -	- - -	- - -	F S 1+
LIVER Lobule						
Central	N 3+	N 2+	N 2+	- - -	d 4+	N Gd 3+
Peripheral	N 4+	N 3+	N 3+	- - -	d 4+	N Gd 4+
Parenchymal cells						
Pericanalicular	N S 4+	N S 3+	N S 2+	- - -	d 4+	N SG 3+
Perinuclear	- - -	F S 3+	d 1+	- - -	- - -	F SG 2+
Kupffer cells	F M 4+	F S 4+	F M 4+	F S 3+	Gd 4+	F SG 3+
Bileduct epithelium						
PANCREAS						
Islet cells	F S 1+	F d 1+	- - -	- - -	- - -	N G 1+
Acinar cells	N S 2+	F d 1+	- - -	- - -	N S 2+	N G 2+
Duct epithelium	F S 1+	F d ±	- - -	- - -	- - -	N G 1+

Table II-17

ANIMAL HAMSTER	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	- - -	F S 2+	N S 3+	N M 4+	F S 1+	N MG 4+
Cords of Billroth	F S ±	- - -	- - -	F S 1+	d ±	N G ±
White pulp						
Macrophages	N S-L 4+	N S-L 4+	N S 3+	N M 4+	d ±	N S 4+
Lymphoid cells	F S 1+	F S 1+	- - -	- - -	- - -	N S 1+
DUODENUM						
Villus, epithelium						
Apex	N S 4+	N Sd 3+	F S 1+	N S 2+	d 4+	N G 4+
Middle	N S 2+	N Sd 2+	F S 1+	N S 2+	d 4+	N G 4+
Crypt	N S 1+	F d 1+	F S 1+	N S 2+	d 4+	N G 3+
Lamina propria macroph.	N S 4+	N S 4+	F S 2+	F M 4+	- - -	F G +
BRAIN						
Neurones Cortical	N S 4+	N S 3+	F d ±	- - -	F S ±	N SG 3+
Purkinje	N S 3+	N S 2+	- - -	- - -	d ±	F G 1+
Glial cells	N S 2+	F S 2+	- - -	- - -	- - -	F S 1+
Endothelial cells	- - -	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	N S 4+	N S 4+	N S 2+	- - -	- - -	N S 4+

Table II-18

ANIMAL FIELD MOUSE	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N S 3+	N S 3+	- - -	F S 2+	d 3+	Gd 3+
Mid portion			- - -			
Distal portion			- - -		d 3+	Gd 2+
Distal tubule	F S ±	F S ±	- - -	- - -	- - -	- - -
Loop of Henle	- - -	- - -	- - -	- - -	- - -	- - -
Collecting tubule						
Principal cells		- - -	- - -	- - -	- - -	- - -
Intercalated cells		F S 2+	- - -	- - -	F S 1+	F S 2+
Glomerulus		- - -	- - -	- - -	- - -	- - -
Visc. epith.	- - -	- - -	- - -	- - -	- - -	- - -
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	- - -	d 2+	d 1+	- - -	d 3+	d 2+
Peripheral	F 1+	d 1+	d 2+	F 2+	d 3+	d 2+
Parenchymal cells						
Pericanalicular	- - -	F S 1+	F S 1+	- - -	Gd 3+	Gd 2+
Perinuclear	- - -	F S 1+	- - -	- - -	Gd 3+	Gd 2+
Kupffer cells	F L 4+	N M 4+	F S 3+	N S 3+	Gd 3+	Gd 2+
Bileduct epithelium	- - -	- - -	- - -	- - -	- - -	F G ±
PANCREAS						
Islet cells	F d 2+	F d ±	- - -	F d ±	- - -	F G 1+
Acinar cells	F S ±	d ±	- - -	- - -	d 2+	N G 2+
Duct epithelium	- - -	- - -	- - -	- - -	- - -	F G 1+

Table II-18

ANIMAL FIELD MOUSE	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N S-L 4+	N M 4+	N M 3+	N M 4+	F M 4+	N LG 4+
Cords of Billroth	F d 1+	F S 2+	- - -	- - -	- - -	N G ±
White pulp						
Macrophages	N Md 4+	F M 4+	F M ±	N M 4+	F Gd 1+	N G 2+
Lymphoid cells	- - -	F S ±	- - -	- - -	- - -	N G +
DUODENUM						
Villus, epithelium						
Apex	N S 1+	F d 1+	F d ±	F S 1+	N Gd 4+	N G 3+
Middle	N S 1+	F d 1+	F d ±	F S 1+	N Gd 4+	N G 3+
Crypt	N S ±	F d ±	- - -	F d ±	N Gd 4+	N G 3+
Lamina propria macroph.	F M 3+	N S 3+	F S 4+	N S 4+	- - -	F G 4+
BRAIN						
Neurones Cortical						
Purkinje						
Glial cells						
Endothelial cells						
Perithelial microglia cells						

Table II-19

ANIMAL MOUSE (AJAX)	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N S-M 4+	N S 4+	F S 1+	F S 1+	d 4+	N G 2+
Mid portion	N S 3+	N S 4+	N S 4+	F S 1+	d 3+	N G 2+
Distal portion	N S 2+	N S 3+	N S 3+	N S 2+	d 4+	N G 4+
Distal tubule	F S 1+	F S 1+	F S 2+	F S ±	d 1+	F G ±
Loop of Henle	F S 1+	- - -	- - -	- - -	- - -	F G ±
Collecting tubule						
Principal cells	F S 1+	F S +	- - -	- - -	- - -	- - -
Intercalated cells	N S 4+	F S 4+	F S 2+	N S 4+	d 2+	F G 1+
Glomerulus	- - -	- - -	- - -	F S 3+	d 1+	N G 1+
Visc. epith.	F S 2+	F d 1+	- - -	- - -	- - -	F G ±
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 2+	N 2+	F 1+	- - -	d 4+	Gd 2+
Peripheral	N 3+	N 3+	N 2+	F 1+	d 4+	Gd 3+
Parenchymal cells						
Pericanalicular	N S 3+	N S 3+	N S 2+	- - -	d 4+	N G 3+
Perinuclear	- - -	F Sd ±	F Sd ±	- - -	d 4+	Gd 3+
Kupffer cells	N S-L 4+	N Sd 4+	- - -	d 1+	d 4+	Gd 3+
Bileduct epithelium	F S 1+	N S 3+	F S 2+	- - -	d 4+	N G ±
PANCREAS						
Islet cells	N S 2+	F Sd 2+	F Sd 1+	F Sd ±	F S ±	F SG 1+
Acinar cells	F S 2+	F S ±	- - -	- - -	d 2+	N G 3+
Duct epithelium	F S 1+	F Sd ±	- - -	- - -	d 4+	N G 1+

Table II-19

ANIMAL MOUSE (AJAX)	E N Z Y M E					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N S-L 4+	N S-L 4+	N S-L 4+	N Sl 3+	F S 3+	N M 4+
Cords of Billroth	- - -	F S ±	F S 1+	- - -	- - -	F Sd 1+
White pulp						
Macrophages	N S-L 4+	N S-L 4+	N S-L 4+	N S 4+	F Sd 1+	N G 3+
Lymphoid cells	- - -	F S ±	F S 1+	- - -	- - -	F G 1+
DUODENUM						
Villus, epithelium						
Apex	F S 2+	N Md 4+	F S ±	F S 1+	N Gd 4+	N G 3+
Middle	F S 2+	N Md 4+	F S ±	F S 1+	N Gd 4+	N G 4+
Crypt	F S 1+	N M 4+	F S ±	F S ±	N Gd 4+	N G 3+
Lamina propria macroph.	F S-L 4+	F S-L 4+	N S-L 4+	F S 1+	F Sd ±	N Gd 2+
BRAIN						
Neurones Cortical	N S 4+	N Sd 4+	N S 3+	F Sd ±	d 1+	F S ±
Purkinje	N S 4+	N Sd 3+	- - -	- - -	d 1+	F S ±
Glial cells	N S 2+	F Sd 1+	- - -	- - -	- - -	- - -
Endothelial cells	- - -	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	N S 2+	N Sd 2+	F S 4+	F S 3+	N S 3+	N S 4+

Table II-20

ANIMAL RAT	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N L 4+	N M 4+	F M 2+	N M 4+	d 4+	Gd 4+
Mid portion					d 4+	Gd 4+
Distal portion	N M 3+	N S 3+	F S 1+	N S 2+	d 4+	Gd 4+
Distal tubule	F S 1+	- - -	- - -	- - -	- - -	- - -
Loop of Henle	F S 1+	F S 1+	- - -	- - -	- - -	F S 1+
Collecting tubule						
Principal cells	N S 1+	F S 1+	- - -	- - -	- - -	- - -
Intercalated cells	N L 4+	N M 2+	F S 1+	F S 2+	d 1+	N S 4+
Glomerulus	- - -	F S 1+	- - -	F S 3+	F S 1+	F S 2+
Visc. epith.	- - -	- - -	F S 1+	- - -	- - -	F S ±
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 3+	N 2+	N 2+	- - -	N Gd 4+	N Gd 3+
Peripheral	N 4+	N 3+	N 3+	- - -	N Gd 4+	N Gd 4+
Parenchymal cells						
Pericanalicular	N Sd 4+	N S 3+	N S 3+	- - -	N Gd 4+	N G 4+
Perinuclear	- - -	F S 1+	- - -	- - -	N Gd 4+	N G 2+
Kupffer cells	N Md 4+	N M 4+	N M 4+	F S ±	N Gd 4+	N G 2+
Bileduct epithelium	F S 1+	F S 1+	F S 1+	- - -	F Gd 2+	F Gd 1+
PANCREAS						
Islet cells	F Gd 2+	d 2+	F S ±	N S 2+	F Gd 1+	N G 2+
Acinar cells	F S ±	F Gd 1+	- - -	- - -	F Gd 2+	N G 1+
Duct epithelium	- - -	d ±	F S ±	F S 1+	- - -	N G 1+

Table II-20

ANIMAL RAT	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N SL 4+	N SL 4+	N M 4+	d 1+	d 2+	N M 4+
Cords of Billroth	F S ±	d 2+	F S 1+	- - -	- - -	F G ±
White pulp						
Macrophages	N S 3+	N M 4+	N M 4+	N S 2+	d 1+	F G 3+
Lymphoid cells	F S ±	d 2+	N S 1+	- - -	- - -	- - -
DUODENUM						
Villus, epithelium						
Apex	N S 3+	N Sd 2+	- - -	F S ±	d 4+	N G 2+
Middle	N S 2+	N Sd 2+	- - -	N S 1+	d 4+	N G 2+
Crypt	N S 1+	M Sd 2+	- - -	N S 1+	d 4+	N G 2+
Lamina propria macroph.	N S 4+	N S 4+	N S 3+	F S 2+	- - -	F S 2+
BRAIN						
Neurones Cortical	F S 1+	d 2+	- - -	F S 2+	- - -	N Sd 4+
Purkinje	F S ±	F Sd 2+	- - -	F S ±	d 2+	F G ±
Glial cells	F S ±	d ±	- - -	N S 2+	d 2+	F G 1+
Endothelial cells	- - -	- - -	- - -	- - -	d 3+	F G 1+
Perithelial microglia cells	N S 4+	N S 4+	N S 3+	N S 4+	d 4+	N S 4+

Table II-21

ANIMAL COW		ENZYME					
TISSUES		APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion	N S 4+	N S 3+	N S 3+	N SM 3+	d 4+	N G 4+
	Mid portion	N S 4+	N S 3+	N S 3+	N S 3+	Gd 4+	N G 4+
	Distal portion	N S 2+	N S 2+	F S ±	F S 1+	Gd 3+	N G 3+
	Distal tubule	- - -	F S ±	- - -	- - -	- - -	- - -
	Loop of Henle	- - -	d 1+	- - -	- - -	- - -	- - -
	Collecting tubule						
	Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
	Intercalated cells	N S 3+	N S 3+	F S 1+	F M 3+	F S 1+	N S 3+
	Glomerulus	F S 2+	- - -	F S 3+	- - -	- - -	- - -
	Visc. epith.	F Sd 3+	- - -	F S ±	F S 1+	- - -	- - -
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	F 1+	F 1+	F 1+	- - -	Gd 4+	G 4+
	Peripheral	F 1+	F 2+	F 2+	- - -	Gd 4+	G 4+
	Parenchymal cells						
	Pericanalicular	F S 1+	F S 1+	F S 3+	- - -	Gd 4+	N G 4+
	Perinuclear	- - -	F S 1+	d 2+	- - -	Gd 4+	N G 4+
	Kupffer cells	N S 4+	N SM 4+	F S 2+	N SM 4+	Gd 4+	N SG 4+
	Bileduct epithelium	- - -	F S ±	- - -	- - -	- - -	- - -
PANCREAS							
	Islet cells	- - -	d 1+	- - -	F Sd 2+	F Sd 1+	N G ±
	Acinar cells	F S 2+	d 1+	- - -	- - -	Gd ±	N G 3+
	Duct epithelium	F S ±	d ±	F S ±	F S -	- - -	N G 2+

Table II-21

ANIMAL COW	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp	N SM 4+					
Macrophages		N SM 4+	N SM 4+	N M 3+	N d 2+	N S 2+
Cords of Billroth	N S 2+	- - -	F S ±	F S 1+	- - -	F G ±
White pulp						
Macrophages	N S 4+	N Sd 3+	N S 1+	F Sd ±	d 1+	N S 3+
Lymphoid cells	- - -	F Sd +	- - -	F M 1+	d 1+	F S 1+
DUODENUM						
Villus,epithelium						
Apex	N S 4+	N S 3+	- - -	- - -	d 4+	N Gd 3+
Middle	N S 3+	N S 3+	- - -	- - -	d 4+	N Gd 3+
Crypt	N S 3+	N S 3+	- - -	- - -	d 4+	N Gd 3+
Lamina propria macroph.	N M 4+	N S 4+	N S 4+	N S 4+	F S 1+	N G 4+
BRAIN						
Neurones Cortical	N S 1+	F S 2+	- - -	- -	d 2+	N Sd 4+
Purkinje	N S 1+	F Sd 2+	- - -	- -	d 2+	F G 1+
Glial cells	F S ±	N S 3+	- - -	- -	- - -	F G 1+
Endothelial cells	- - -	- - -	- - -	- -	F Sd ±	F G 1+
Perithelial microglia cells	N S 3+	N S 3+	d ±	F S	F S 3+	F G 1+

Table II-22

ANIMAL SHEEP	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N SM 4+	N SL 4+	F S 4+	N S 3+	N S-L 2+	- - -
Cords of Billroth	F S 1+	F S 1+	- - -	F S 2+	F Sd 1+	- - -
White pulp						
Macrophages	- - -	F S 1+	- - -	- - -	- - -	F G ±
Lymphoid cells	- - -	- - -	- - -	- - -	- - -	- - -
DUODENUM						
Villus,epithelium						
Apex					d 4+	N G 2+
Middle					d -	N G 1+
Crypt					d -	N G 1+
Lamina propria macroph.					F Sd 1+	F G ±
BRAIN						
Neurones Cortical	- - -	F S 1+	- - -	- - -	N Gd 2+	- - -
Purkinje	- - -	- - -	- - -	- - -	d +	- - -
Glial cells	- - -	- - -	- - -	- - -	F S ±	- - -
Endothelial cells	- - -	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	- - -	F S 2+	- - 1+	F S ±	F S 2+	F S 1+

Table II-23

ANIMAL HORSE	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N SM 4+	N SL 4+	F SM 3+	F SM 4+	d 2+	N G 2+
Cords of Billroth	- - -	- - -	- - -	- - -	d 2+	N G ±
White pulp						
Macrophages	N SM 4+	N SL 4+	F SL 3+	- - -	N SL 4+	N SL 4+
Lymphoid cells	- - -	- - -	- - -	- - -	- - -	F G ±
DUODENUM						
Villus, epithelium						
Apex		N S 4+	F S ±	F S ±	d 4+	N G 4+
Middle		N S 4+	F S ±	F S ±	d -	N G 4+
Crypt		N S 4+	F S ±	- - -	d 4+	N G 4+
Lamina propria macroph.		N M 4+	F S 3+	N SM 4+	F SM 4+	N M 4+
BRAIN						
Neurones Cortical	F S ±	- - -	- - -	- - -	d 3+	N S 2+
Purkinje	N S 2+	- - -	- - -	- - -	d 3+	N M 2+
Glial cells	- - -	F Sd ±	- - -	- - -	d 1+	F S 1+
Endothelial cells	- - -	- - -	- - -	- - -	F Sd 1+	- - -
Perithelial microglia cells	- - -	F Sd 1+	- - -	F S 3+	F Sd 3+	N M 4+

Table II-23

ANIMAL HORSE	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N S-M 4+	N M 4+	N S 2+	N S 4+	d 4+	N SM 4+
Mid portion						
Distal portion		N S 2+			d 4+	N G 2+
Distal tubule	F S 1+	- - -	N S 1+	- - -	- - -	- - -
Loop of Henle	- - -	- - -	N S 1+	- - -	d 1+	N G 1+
Collecting tubule						
Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
Intercalated cells	N M 3+	N S 3+	N S 2+	N S 2+	d 1+	N S 4+
Glomerulus	N S 1+	- - -	F S 1+	- - -	- - -	F S 1+
Visc. epith.	- - -	- - -	F S 1+	- - -	F S 1+	F G 1+
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 4+	N 3+	N 2+	N 1+	N 4+	N 4+
Peripheral	N 3+	N 4+	N 3+	N 1+	N 4+	N 3+
Parenchymal cells						
Pericanalicular	N S 4+	N S 4+	N S 2+	N S 1+	N S 4+	N Gd 4+
Perinuclear	- - -	F S 1+	- - -	- - -	N S 2+	N Gd 3+
Kupffer cells	F L 4+	F M 4+	N S 3+	F S 2+	N Gd 3+	N Gd 3+
Bileduct epithelium	N S 1+	N S 1+	- - -	- - -	Gd 2+	N G 4+
PANCREAS						
Islet cells	- - -	F Sd ±	- - -	- - -	F SG 1+	N G 1+
Acinar cells	N S 3+	F Sd 1+	- - -	- - -	N Gd 3+	N G 2+
Duct epithelium	N S 2+	- - -	F S 1+	F S ±	d 2+	N SG 3+

Table II-24

ANIMAL PIG		E N Z Y M E					
TISSUES		APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion	N SM 4+	N S 4+	- - -	N S 4+	d 4+	N G 4+
	Mid portion						
	Distal portion	N S 2+	N S 2+			d 4+	N G 1+
	Distal tubule	F S ±	- - -	- - -	- - -	- - -	- - -
	Loop of Henle	- - -	d 1+	F S 1+	- - -	d 1+	N G 1+
	Collecting tubule						
	Principal cells	N S 3+	- - -	- - -	- - -	- - -	- - -
	Intercalated cells	N S 4+	N S 2+	- - -	F S 2+	d 2+	N S 2+
	Glomerulus	N S 4+	d 1+	- - -	F S 2+	- - -	F S 1+
	Visc. epith.	- - -	- - -	- - -	- - -	d 3+	F M 1+
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	N 4+	N 3+	F 1+	F 1+	d 4+	d 4+
	Peripheral	N 3+	N 2+	F 2+	F 1+	d 4+	d 4+
	Parenchymal cells						
	Pericanalicular	N S 4+	N S 3+	F S 1+	N S ±	Gd 4+	N Gd 4+
	Perinuclear	- - -	F S 1+	- - -	- - -	Gd 4+	N Gd 4+
	Kupffer cells	N SM 4+	N SM 4+	F S ±	F S 4+	N G 4+	N Gd 4+
	Bileduct epithelium	F S 1+	F S ±	- - -	- - -	Gd 3+	N G 2+
PANCREAS							
	Islet cells	F S ±	d ±	- - -	- - -	F Sd 1+	F G 1+
	Acinar cells	F S ±	d ±	- - -	- - -	F Sd 1+	N G 3+
	Duct epithelium	- - -	d ±	- - -	F S ±	- - -	N G 2+

Table II-24

ANIMAL PIG	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N SM 4+	N SM 4+	F SM 2+	N S 2+	F Sd 2+	F S 2+
Cords of Billroth	- - -	F Sd +	- - -	- - -	- - -	F G -
White pulp						
Macrophages	N SM 4+	F S 2+	F SM 2+	F S 3+	F S 1+	F S 2+
Lymphoid cells	- - -	F S 2+	- - -	- - -	d 1+	F G 1+
DUODENUM						
Villus,epithelium						
Apex	N S 3+	N S 2+	- - -	- - -	d 4+	N Gd 3+
Middle	N S 4+	N S 2+	- - -	- - -	d 3+	N G 2+
Crypt	N S 2+	N S 2+	- - -	- - -	d 3+	N G 2+
Lamina propria macroph.	N M 4+	N S 3+	F M 3+	F S 3+	- - -	N G 2+
BRAIN						
Neurones Cortical	N S 3+	F Sd +	- - -	- - -	- - -	N S 2+
Purkinje	F S ±	F Sd +	- - -	- - -	d 1+	F Sd ±
Glial cells	N S 2+	F S ±	- - -	- - -	- - -	F S 2+
Endothelial cells	- - -	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	N S 2+	F S 2+	F S 2+	F S 1+	F S 3+	N S 2+

Table II-25

ANIMAL CAT		ENZYME					
TISSUES		APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion	F S 2+	N S 2+	N S 2+	N SM 3+	d 4+	Gd 4+
	Mid portion						
	Distal portion					d 3+	Gd 3+
	Distal tubule	F S 1+	F S ±	- - -	- - -	- - -	- - -
	Loop of Henle	- - -	- - -	- - -	- - -	- - -	- - -
	Collecting tubule						
	Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
	Intercalated cells	- - -	N SM 3+	- - -	N SM 4+	d 2+	- - -
	Glomerulus	- - -	- - -	- - -	N S 3+	- - -	- - -
	Visc. epith.	F S 1+	- - -	- - -	- - -	- - -	- - -
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	N 3+	N 3+	N 2+	N 2+	d 4+	N 2+
	Peripheral	N 2+	N 2+	N 3+	N 2+	d 3+	N 4+
	Parenchymal cells						
	Pericanalicular	N S 3+	N S 3+	N S 2+	N S 2+	N SG 4+	N Gd 3+
	Perinuclear	- - -	F S 1+	- - -	F S ±	F SG 2+	N SG 2+
	Kupffer cells	F SL 4+	F SL 4+	F SL 4+	F SL 4+	N SG 3+	N SG 4+
	Bileduct epithelium	F S 1+	F S 1+	- - -	F S ±	F SG 1+	F S ±
PANCREAS							
	Islet cells	F S 2+	d ±	d ±	F S 2+	- - -	N G ±
	Acinar cells	F S ±	d ±	- - -	- - -	d ±	N G 2+
	Duct epithelium	- - -	- - -	- - -	N Sd 2+	d 4+	N G 1+

Table II-25

ANIMAL CAT	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	F S 4+	N SM 4+	F SL 4+	N S 2+	F G 1+	F G 2+
Cords of Billroth	F S 1+	F Sd ±	F S 2+	F S ±	- - -	- - -
White pulp						
Macrophages	F S ±	F Sd 1+	N M 4+	N M 3+	F G 1+	F G 2+
Lymphoid cells	F S 2+	- - -	F S 1+	F S 2+	- - -	F G ±
DUODENUM						
Villus,epithelium						
Apex	F S 1±	F Sd 2+	- - -	N S 3+	- - -	N G ±
Middle	F S ±	F Sd 2+	- - -	N S 3+	- - -	N G ±
Crypt	F S ±	F Sd 2+	- - -	N S 3+	- - -	N G ±
Lamina propria macroph.	- - -	F S 3+	N Sd 4+	F M 3+	F SG 2+	F G 1+
BRAIN						
Neurones Cortical	F S ±	F Sd 2+	F S 1+	d 1+	d 2+	N G 1+
Purkinje	F Sd ±	F Sd 1+	F Sd ±	d ±	d 2+	F G ±
Glial cells	F Sd 1+	- - -	F S 1+	F Sd ±	- - -	- - -
Endothelial cells	- - -	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	- - -	- - -	F S 3+	N S 3+	F S 2+	N G 2+

Table II-26

ANIMAL DOG	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N SM 4+	N SM 4+	N SM 4+	N SM 4+	d 4+	Gd 4+
Mid portion						
Distal portion	N S 4+	F S 2+	F S 2+		d 2+	Gd 3+
Distal tubule	- - -	- - -	- - -	- - -	- - -	- - -
Loop of Henle	F S 1+	- - -	- - -	- - -	- - -	- - -
Collecting tubule						
Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
Intercalated cells	F S 2+	F S 2+	F S 2+	F S 1+	- - -	d 1+
Glomerulus	- - -	- - -	F S 2+	F S 3+	- - -	F S 1+
Visc. epith.	F S 2+	- - -	- - -	- - -	- - -	- - -
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 2+	N 2+	F 1+	F 1+	N 4+	N 3+
Peripheral	N 3+	N 3+	F 3+	F 2+	N 4+	N 4+
Parenchymal cells						
Pericanalicular	N S 3+	N S 3+	F S 1+	F S 2+	N S 4+	N S 4+
Perinuclear	F S 1+	F S 1+	F S 1+	F S 1+	N S 4+	N S 4+
Kupffer cells	F M 4+	N SL 4+	N SL 4+	F SL 4+	N S 4+	N S 4+
Bileduct epithelium	F S 2+	F S ±	N S 3+	N S 3+	F M 2+	F S 1+
PANCREAS						
Islet cells	F S ±	d 1+	- - -	F Sd 1+	N G 1+	N G ±
Acinar cells	F S ±	F Sd 1+	- - -	- - -	N G 3+	N G 2+
Duct epithelium	F S 1+	N SG 2+	N SG 2+	N Sd 2+	Gd 1+	F Gd 1+

Table II-26

ANIMAL DOG	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages						
Cords of Billroth						
White pulp						
Macrophages						
Lymphoid cells						
DUODENUM						
Villus, epithelium						
Apex	F S 1+	F Sd 1+	- - -	F S ±	F Gd ±	N G ±
Middle	F S 1+	F Sd 1+	- - -	F S ±	F Gd ±	N G ±
Crypt	F S 1+	F Sd 1+	- - -	F S ±	F Gd ±	N G ±
Lamina propria macroph.	F S ±	F Sd 3+	F Sd 3+	F SL 4+	F Gd ±	N G ±
BRAIN						
Neurones Cortical						
Purkinje						
Glial cells						
Endothelial cells						
Perithelial microglia cells						

Table II-27

ANIMAL	MAN I (CHILD)	ENZYME																	
		APase Gomori			APase Barka			Glucuronidase			Glucosaminidase			N-Acetate Esterase			N-AS Acet Esterase		
TISSUES		No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.
KIDNEY	Prox. tubule																		
	Prox. portion																		
	Mid portion																		
	Distal portion																		
	Distal tubule																		
	Loop of Henle																		
	Collecting tubule																		
	Principal cells																		
	Intercalated cells																		
	Glomerulus																		
	Visc. epith.																		
	Parietal epith.																		
	Endothelium																		
LIVER	Lobule																		
	Central	N		3+	N		3+	N		3+	N		1+	N	G	4+	N		2+
	Peripheral	N		4+	N		4+	N		3+	N		2+	N	G	4+	N		2+
	Parenchymal cells																		
	Pericanalicular	N	SM	3+	N	SM	4+	N	S	4+	N	S	±	N	SG	4+	N	G	2+
	Perinuclear	-	-	-	-	-	-	-	-	-	-	-	-	N	G	4+	N	G	2+
	Kupffer cells	N	Sd	4+	N	SM	4+	N	S	2+	N	S	2+	N	G	3+	N	S	4+
	Bileduct epithelium	F	S	1+	F	S	1+	F	S	1+	F	S	3+	F	S	2+	N	G	±
PANCREAS																			
	Islet cells	F	Sd	2+	N	S	3+	-	-	-	-	-	-	F	G	1+	N	G	1+
	Acinar cells	N	SM	4+	N	S	4+	F	S	3+	-	-	-		d	4+	N	Gd	4+
	Duct epithelium	F	S	±	F	S	1+	F	S	2+	F	S	±	N	Gd	4+	N	Gd	4+

Table II-27

ANIMAL MAN I (CHILD)	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N SM 3+	N SM 3+	N SM 3+	F S 2+	N Gd 2+	N S 1+
Cords of Billroth	F S 1+	F S 2+	F S ±	- - -	F G 1+	- - -
White pulp						
Macrophages	F SM 3+	F SM 3+	F Sd 2+	F S 2+	F Gd 1+	F G ±
Lymphoid cells	F S ±	- - -	- - -	- - -	- - -	- - -
DUODENUM						
Villus,epithelium						
Apex	F S 2+	F S 2+	- - -	- - -	d 4+	N G 3+
Middle	F S 2+	F S 2+	- - -	- - -	d 4+	N G 3+
Crypt	F S 1+	F S 1+	- - -	- - -	d 4+	N G 3+
Lamina propria macroph.	F S 1+	F Sd 1+	F Sd 1+	F S 1+	d 2+	N G 3+
BRAIN						
Neurones Cortical	N S 2+	N Sd 3+	- - -	- - -	F Gd 2+	F G ±
Purkinje	N S 2+	N Sd 2+	- - -	- - -	F Gd 2+	- - -
Glial cells	F S 2+	F S 2+	- - -	- - -	- - -	- - -
Endothelial cells	- - -	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	F S 2+	F S 2+	F S ±	F S ±	N SM 4+	N G 4+

Table II-28

ANIMAL MAN II (ADULT)	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N S 2+	F S 1+	N S 3+	F S 3+	Gd 3+	N G 4+
Mid portion	F S 1+	F S 1+	F S 2+	F S 2+	Gd 3+	N G 2+
Distal portion	F S 1+	F S ±	F S 2+	F S 1+	Gd 3+	N G 1+
Distal tubule	- - -	- - -	- - -	- - -	- - -	- - -
Loop of Henle	F S ±	- - -	- - -	- - -	F S 2+	F G ±
Collecting tubule						
Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
Intercalated cells	F S ±	F Sd ±	F S 1+	F S 1+	F S 2+	F S 4+
Glomerulus	- - -	- - -	F S ±	- - -	F S 2+	F S 1+
Visc. epith.	- - -	- - -	- - -	- - -	N S 2+	N G 1+
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N - 2+	N 2+	N 1+	N 1+	N G 3+	N 2+
Peripheral	N 2+	N 2+	N 2+	N 2+	N G 3+	N 3+
Parenchymal cells						
Pericanalicular	N SM 2+	N SM 2+	N S 3+	N S 2+	N G 3+	N G 3+
Perinuclear	- - -	- - -	- - -	- - -	N G 3+	N G 3+
Kupffer cells	N S 3+	N SM 2+	F S 1+	N S ±	N G 3+	N G 4+
Bileduct epithelium	N S 1+	- - -	F S 1+	F S 1+	F S 2+	N G ±
PANCREAS						
Islet cells	F S 2+	N S 2+	- - -	- - -	N G ±	N G 1+
Acinar cells	N S 4+	N S 2+	- - -	- - -	N Gd 4+	N Gd 4+
Duct epithelium	F S ±	F S ±	- - -	F S ±	N Gd 4+	N Gd 4+

Table II-28

ANIMAL MAN II (ADULT)	E N Z Y M E					
TISSUE	APase Gomori No.Si.St.	APase Darka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	F S 3+	F Sd 2+	F S 3+	- - -	F G 3+	N G 2+
Cords of Billroth	- - -	- - -	- - -	- - -	- - -	- - -
White pulp						
Macrophages	F S 2+	F Sd 2+	F S 3+	- - -	F G 2+	F G ±
Lymphoid cells	- - -	- - -	- - -	- - -	- - -	- - -
DUODENUM						
Villus,epithelium						
Apex		- - -	- - -	- - -		
Middle		- - -	- - -	- - -		
Crypt		- - -	- - -	- - -		
Lamina propria macroph.		- - -	F Sd 1+	F S 1+		
BRAIN						
Neurones Cortical						
Purkinje						
Glial cells						
Endothelial cells						
Perithelial microglia cells						

TABLE III.

Modulations of Lysosomal Enzymes by Experimental Means.

Table III-1 to Table III-27 inclusive.

EXP. I PHENACETIN RATS

Table III-1

ANIMAL	RAT I	ENZYME					
TISSUES		APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion	N SM 4+	N M 4+	N SM 1+	N SM 2+	d 4+	Gd 3+
	Mid portion	N M 4+	N S 3+	N SM 1+	N S 2+	d 4+	Gd 3+
	Distal portion	N S 2+	N S 1+	F S ±	F S 1+	d 4+	Gd 3+
	Distal tubule	- - -	- - -	- - -	- - -	- - -	- - -
	Loop of Henle	- - -	d 1+	- - -	- - -	- - -	F S 1+
	Collecting tubule						
	Principal cells	N S 2+	F S 1+	- - -	- - -	- - -	- - -
	Intercalated cells	N M 4+	F M 2+	F S 1+	F S 2+	d 1+	N S 4+
	Glomerulus	- - -	F S 1+	- - -	F S 3+	F S 1+	F S 2+
	Visc. epith.	- - -	- - -	F S 1+	- - -	- - -	F S ±
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	d 1+	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	N 2+	N 2+	F 1+	- - -	d 3+	d 3+
	Peripheral	N 3+	N 3+	N 2+	F 1+	d 3+	d 2+
	Parenchymal cells						
	Pericanalicular	N S 4+	N SM 3+	N SM 2+	F S ±	d 3+	F S 2+
	Perinuclear	- - -	- - -	- - -	- - -	d 3+	Gd 2+
	Kupffer cells	N M 4+	F M 4+	N SM 4+	- - -	d 3+	N M 4+
	Bileduct epithelium	- - -	- - -	- - -	- - -	F S 1+	F S 1+

EXP. I PHENACETIN

Table III-2

ANIMAL RAT II		ENZYME					
TISSUES		APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion	N SM 4+	N SM 4+	N SM 1+	N SM 2+	d 4+	Gd 3+
	Mid portion	N M 4+	N M 4+	N M 1+	N S 2+	d 4+	Gd 3+
	Distal portion	N S 2+	N S 2+	F S ±	F S 1+	d 4+	Gd 3+
	Distal tubule	- - -	- - -	- - -	- - -	- - -	- - -
	Loop of Henle	- - -	- - -	- - -	- - -	- - -	F S 1+
	Collecting tubule						
	Principal cells	N S 2+	N S 2+	- - -	- - -	- - -	- - -
	Intercalated cells	N M 4+	N M 4+	F S 1+	N S 4+	d 1+	N S 4+
	Glomerulus	- - -	- - -	- - -	F S 3+	F S 1+	F S 2+
	Visc. epith.	- - -	- - -	F S 1+	- - -	- - -	F S ±
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	N 2+	N 2+	F 1+	- - -	d 4+	d 3+
	Peripheral	N 3+	N 3+	N 2+	F 1+	d 4+	d 2+
	Parenchymal cells						
	Pericanalicular	N S 4+	N SM 3+	N S 2+	F S 1+	d 4+	F SM 2+
	Perinuclear	F S ±	- - -	- - -	- - -	d 2+	Gd 2+
	Kupffer cells	N M 4+	F M 4+	N SM 4+	- - -	d 4+	F Sd 3+
	Bileduct epithelium	- - -	- - -	- - -	- - -	F Sd 1+	F S 1+

EXP. I PHENACETIN

Table III-3

ANIMAL	RAT III	ENZYME																	
	CONTROL	APase Gomori			APase Barka			Glucuron-idase			Glucos-aminidase			N-Acetate Esterase			N-AS Acet Esterase		
TISSUES		No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.
KIDNEY	Prox. tubule																		
	Prox. portion	N	S	4+	N	M	4+	F	M	1+	N	M	3+		d	4+		Gd	4+
	Mid portion	N	M	4+	N	S	3+	N	SM	1+	N	S	3+		d	4+		Gd	3+
	Distal portion	N	S	2+	N	S	2+	F	S	±	F	S	2+		d	4+		Gd	3+
	Distal tubule	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Loop of Henle	-	-	-	-	d	1+	-	-	-	-	-	-	-	-	-	F	S	1+
	Collecting tubule																		
	Principal cells	N	S	2+	F	SM	1+	-	-	-	-	-	-	-	-	-	-	-	-
	Intercalated cells	N	M	4+	N	M	2+	F	S	1+	F	SM	2+		d	1+	N	S	4+
	Glomerulus	-	-	-	F	S	1+	-	-	-	F	S	3+	F	S	1+	F	S	1+
	Visc. epith.	-	-	-	-	-	-	F	S	1+	-	-	-	-	-	-	F	S	±
	Parietal epith.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Endothelium	-	-	-		d	1+	-	-	-	-	-	-	-	-	-	-	-	-
LIVER	Lobule																		
	Central	N		3+	N		3+	F		1+	F	S	±		d	4+		d	4+
	Peripheral	N		4+	N		4+	N		3+	N		2+		d	4+	N	Gd	3+
	Parenchymal cells																		
	Pericanalicular	N	SM	4+	N	M	4+	N	SM	3+	N	S	2+		d	4+	N	SM	4+
	Perinuclear	-	-	-	-	-	-	-	-	-	-	-	-		d	4+		Gd	2+
	Kupffer cells	N	SM	4+	F	ML	4+	F	S	±		d	4+		d	4+	N	Gd	4+
	Bileduct epithelium	F	S	1+	F	S	1+	-	-	-	-	-	-	F	Sd	1+	F	S	1+

EXP. II CORTISONE

Table III-4

ANIMAL RAT IV		E N Z Y M E					
TISSUES		APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion	N SM 4+	N S 4+	F SM 1+	N M 3+	d 4+	Gd 4+
	Mid portion	N M 4+	N SM 3+	N SM 1+	N S 3+	d 4+	Gd 3+
	Distal portion	N S 3+	N S 2+	F S ±	F S 1+	d 4+	Gd 3+
	Distal tubule	- - -	- - -	- - -	- - -	- - -	- - -
	Loop of Henle	- - -	F S ±	- - -	F S 1+	- - -	F S 1+
	Collecting tubule						
	Principal cells	F S 1+	F Sd 1+	- - -	- - -	- - -	- - -
	Intercalated cells	N S 4+	F S 2+	F S 2+	F S 3+	d 1+	N S 4+
	Glomerulus	- - -	F Sd 1+	- - -	F S 3+	F S 1+	F S 2+
	Visc. epith.	- - -	- - -	F S 1+	- - -	- - -	- - -
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	d 1+	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	N 4+	N 3+	F 1+	- - -	d 4+	d 3+
	Peripheral	N 4+	N 4+	N 3+	F ±	d 3+	Gd 2+
	Parenchymal cells						
	Pericanalicular	N SM 4+	N SM 4+	N S 2+	F S ±	d 4+	N Gd 4+
	Perinuclear	- - -	- - -	- - -	- - -	d 2+	Gd 2+
	Kupffer cells	N M 4+	F L 4+	N ML 4+	F S 1+	d 4+	Gd 3+
	Bileduct epithelium	F S 1+	- - -	F S 1+	- - -	F Sd 1+	F S 1+

EXP. II CORTISONE

Table III-5

ANIMAL RAT V	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N SM 4+	N S 4+	F SM 2+	N M 3+	d 4+	Gd 4+
Mid portion	N SM 4+	N SM 3+	N SM 1+	N S 3+	d 4+	Gd 3+
Distal portion	N S 3+	N S 2+	F S ±	F S 1+	d 4+	Gd 3+
Distal tubule	- - -	- - -	- - -	- - -	- - -	- - -
Loop of Henle	- - -	F S ±	- - -	- - -	- - -	F S 1+
Collecting tubule						
Principal cells	F S 1+	d 1+	- - -	- - -	- - -	- - -
Intercalated cells	N S 4+	F S 2+	F S 2+	F S 3+	d 1+	N SM 4+
Glomerulus	- - -	d 1+	- - -	F S 3+	F S 1+	F S 2+
Visc. epith.	- - -	- -	F S 1+	- - -	- - -	- - -
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	d 1+	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 4+	N 3+	F 1+	- - -	d 4+	d 3+
Peripheral	N 4+	N 4+	F 2+	F 1+	d 2+	Gd 2+
Parenchymal cells						
Pericanalicular	N SM 4+	N M 4+	F S 1+	F S ±	d 4+	Gd 2+
Perinuclear	- - -	- - -	- - -	- - -	d 4+	Gd 1+
Kupffer cells	N M 4+	F ML 4+	N ML 4+	F S ±	d 4+	Gd 4+
Bileduct epithelium	F S 1+	- - -	F S 1+	- - -	F Sd 1+	F Sd 1+

EXP. II CORTISONE

Table III-6

ANIMAL	RAT VI	ENZYME					
	CONTROL	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
TISSUES							
KIDNEY	Prox. tubule						
	Prox. portion	N SM 4+	N S 4+	F M 1+	N M 3+	d 4+	Gd 4+
	Mid portion	N SM 4+	N SM 3+	N SM 1+	N SM 3+	d 4+	Gd 3+
	Distal portion	N S 3+	N S 2+	F S ±	F S 1+	d 4+	Gd 3+
	Distal tubule	- - -	- - -	- - -	- - -	- - -	- - -
	Loop of Henle	F S 1+	F S ±	- - -	- - -	- - -	F S 1+
	Collecting tubule						
	Principal cells	N S 1+	F S 1+	- - -	- - -	- - -	- - -
	Intercalated cells	N S 3+	F SM 2+	F S 1+	F SM 3+	d 1+	N G 4+
	Glomerulus	- - -	F S 1+	- - -	F S 3+	F S 1+	F S 2+
	Visc. epith.	- - -	- - -	F S 1+	- - -	- - -	F S ±
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	d 1+	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	N 3+	N 3+	F 1+	F ±	d 4+	d 3+
	Peripheral	N 4+	N 4+	N 3+	N 2+	d 4+	d 2+
	Parenchymal cells						
	Pericanalicular	N SM 4+	N SM 4+	N SM 3+	N S 2+	d 4+	Gd 2+
	Perinuclear	- - -	- - -	- - -	- - -	d 4+	Gd 3+
	Kupffer cells	N SM 4+	F SL 4+	N SM 4+	F S ±	d 4+	F Gd 3+
	Bileduct epithelium	F S 1+	F S 1+	F S 1+	- - -	F Sd 1+	F G 1+

EXP. 4 GLUCAGON EXP. V PHOSPHORUS

Table III-7

ANIMAL RAT 1	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N S 3+	N Sd 2+	N S 2+	N M 3+	N d 4+	N G 4+
Mid portion	N M 4+	N M 4+	N S 2+	N M 3+	d 4+	N G 4+
Distal portion	N S 1+	N Sd 1+	F S ±	F M ±	d 4+	N G 1+
Distal tubule	- - -	- - -	- - -	- - -	- - -	- - -
Loop of Henle	- - -	- - -	- - -	- - -	- - -	- - -
Collecting tubule						
Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
Intercalated cells	N S 3+	F S 2+	d ±	N S 3+	N S 1+	N S 3+
Glomerulus	F S 2+	N Sd 1+	d ±	N S 3+	N S 1+	N S 3+
Visc. epith.	- - -	- - -	- - -	- - -	- - -	- - -
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 2+	N 1+	N 1+	- - -	d 4+	N 1+
Peripheral	N 4+	N 3+	N 2+	F 2+	d 4+	N 2+
Parenchymal cells						
Pericanalicular	N SM 4+	N S 3+	N S 2+	F S 2+	d 4+	N SG 4+
Perinuclear	- - -	- - -	F Sd ±	- - -	d 4+	F G 1+
Kupffer cells	N SM 4+	N S 4+	F SM 4+	- - -	d 4+	N S 4+
Bileduct epithelium	N S 2+	N S 2+	- - -	- - -	d 1+	N G 1+

Table III-8

EXP. 4 GLUCAGON

ANIMAL RAT 2		ENZYME					
TISSUES		APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion	N S 3+	N Sd 2+	N S 2+	N M 3+	d 4+	N MG 4+
	Mid portion	N M 4+	N S 2+	N S 2+	N M 3+	d 4+	N G 4+
	Distal portion	N S 1+	N Sd 1+	F S ±	F M ±	d 4+	N G 1+
	Distal tubule	- - -	- - -	- - -	- - -	- - -	- - -
	Loop of Henle	- - -	- - -	- - -	- - -	- - -	- - -
	Collecting tubule						
	Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
	Intercalated cells	N S 3+	F S 2+	d ±	N S 3+	N S 1+	N S 4+
	Glomerulus	F S 2+	d 1+	d ±	N S 3+	N S 1+	N S 3+
	Visc. epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	N 2+	N 1+	N 1+	- - -	d 4+	N 2+
	Peripheral	N 4+	N 3+	N 2+	F 2+	d 4+	N 3+
	Parenchymal cells						
	Pericanalicular	N SM 4+	N S 3+	N S 2+	F S 2+	d 4+	N S 4+
	Perinuclear	- - -	- - -	F Sd ±	- - -	d 4+	F G 1+
	Kupffer cells	N SM 4+	N S 4+	F SM 4+	- - -	d 4+	N S 4+
	Bileduct epithelium	N S 2+	N S 2+	- - -	- - -	d 1+	N G 1+

EXP. 4 GLUCAGON EXP. V PHOSPHORUS CONTROL

Table III-9

ANIMAL RAT 3	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N S 3+	N Sd 2+	N S 2+	N M 3+	d 4+	N Gd 4+
Mid portion	N M 4+	N M 4+	N S 2+	N M 3+	d 4+	N Gd 4+
Distal portion	N S 1+	N Sd 1+	F S ±	F M ±	d 4+	N Gd 1+
Distal tubule	- - -	- - -	- - -	- - -	- - -	- - -
Loop of Henle	- - -	- - -	- - -	- - -	- - -	- - -
Collecting tubule						
Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
Intercalated cells	N S 3+	F S 2+	d ±	N S 3+	N S 1+	F Gd 2+
Glomerulus	F S 2+	d 1+	d ±	N S 1+	N S 1+	F S 2+
Visc. epith.	- - -	- - -	- - -	- - -	- - -	- - -
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 2+	N 2+	N 1+	- - -	d 4+	N 2+
Peripheral	N 4+	N 4+	N 2+	F 1+	d 4+	N 3+
Parenchymal cells						
Pericanalicular	N S 4+	N S 3+	N S 2+	d 1+	d 4+	N G 4+
Perinuclear	- - -	- - -	F Sd ±	- - -	d 4+	N G 2+
Kupffer cells	N SM 4+	N S 4+	F S 3+	F S 1+	d 4+	N MG 4+
Bileduct epithelium	N S 2+	N S 2+	- - -	- - -	d 1+	N G 1+

EXP. 5 PHOSPHORUS

Table III-10

ANIMAL RAT 1	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N SM 3+	N M 2+	- - -	N M 3+	d 4+	N Gd 3+
Mid portion	N M 4+	N M 4+	F M 1+	N Md 3+	d 4+	N G 4+
Distal portion	N S 1+	N Sd 1+	- - -	F S 1+	d 4+	N Gd 1+
Distal tubule	- - -	- - -	- - -	- - -	- - -	- - -
Loop of Henle	- - -	- - -	- - -	- - -	- - -	- - -
Collecting tubule						
Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
Intercalated cells	N S 3+	F S 2+	- - -	F S 4+	d 1+	N S 3+
Glomerulus	F S 2+	d 1+	- - -	- - -	d 1+	F G 1+
Visc. epith.	- - -	- - -	- - -	- - -	- - -	F G ±
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 2+	N 2+	N 1+	- - -	d 4+	N 3+
Peripheral	N 4+	N 3+	N 2+	F 1+	d 4+	N 2+
Parenchymal cells						
Pericanalicular	N S 4+	N S 3+	N S 2+	F Sd 1+	d 4+	N SG 4+
Perinuclear	- - -	- - -	- - -	- - -	d 4+	N G 2+
Kupffer cells	F SM 4+	- - -	F S 3+	F S 1+	d 4+	N S 4+
Bileduct epithelium	N S 2+	- - -	- - -	- - -	d 1+	N G 1+

EXP. 6 TESTOSTERONE

Table III-11

ANIMAL MOUSE 1	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N S 4+	N S 4+	N Sd 40	F S 1+	d 2+	N G 2+
Mid portion	N S 2+	N S 3+	N Sd 40	F S 1+	d 3+	N G 2+
Distal portion	N S 2+	N S 3+	N Sd 40	N S 4+	d 4+	N G 4+
Distal tubule	F S 1+	F S 1+	d ±	F S 1+	d 1+	F G ±
Loop of Henle	F S 1+	d ±	- - -	- - -	- - -	F G ±
Collecting tubule						
Principal cells	F S 2+	F S 1+	- - -	- - -	- - -	- - -
Intercalated cells	N S 4+	F S 4+	N S 4+	N S 4+	d 2+	F G 1+
Glomerulus	F S 1+	- - -	- - -	F S 3+	d 1+	N G 1+
Visc. epith.	F S 1+	- - -	- - -	- - -	- - -	F G ±
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 1+	N 1+	- - -	- - -	d 4+	N G 2+
Peripheral	N 2+	N 2+	F 1+	F 1+	d 4+	N G 2+
Parenchymal cells						
Pericanalicular	F S 3+	N S 3+	d ±	- - -	d 4+	N G 3+
Perinuclear	- - -	F Sd ±	- - -	- - -	d 4+	N G 2+
Kupffer cells	N S 3+	N Sd 4+	F S 1+	Sd 1+	d 4+	N G 3+
Bileduct epithelium	F S 1+	N S 3+	- - -	- - -	d 4+	N G ±

EXP. 6 TESTOSTERONE

Table III-12

ANIMAL MOUSE 2		E N Z Y M E					
TISSUES		APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion	N S 4+	N S 4+	N Sd 40	F S 1+	d 2+	N G 2+
	Mid portion	N S 3+	N S 3+	N Sd 40	F S 1+	d 3+	N G 2+
	Distal portion	N S 2+	N S 3+	N Sd 40	N S 4+	d 4+	N G 4+
	Distal tubule	F S 1+	F S 1+	d ±	F S 1+	d 1+	F G ±
	Loop of Henle	F S 1+	d ±	- - -	- - -	- - -	F G ±
	Collecting tubule						
	Principal cells	N S 2+	F S 1+	- - -	- - -	- - -	- - -
	Intercalated cells	N S 4+	F S 4+	N S 4+	N S 4+	d 2+	F G 1+
	Glomerulus	F S 1+	- - -	- - -	F S 3+	d 1+	N G 1+
	Visc. epith.	F S 1+	- - -	- - -	- - -	- - -	F G ±
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	N 2+	N 1+	- - -	- - -	d 4+	N G 2+
	Peripheral	N 3+	N 2+	F 1+	- - -	d 4+	N G 2+
	Parenchymal cells						
	Pericanalicular	N S 4+	N S 3+	d ±	- - -	d 4+	N Gd 3+
	Perinuclear	- - -	d ±	- - -	- - -	d 4+	N Gd 2+
	Kupffer cells	N S 4+	N S 4+	F Sd 2+	d 1+	d 4+	N Gd 3+
	Bileduct epithelium	F S 1+	N S 3+	- - -	- - -	d 4+	N G ±

EXP. 6 TESTOSTERONE

Table III-13

ANIMAL	MOUSE 3	ENZYME																	
TISSUES		APase Gomori			APase Barka			Glucuron-idase			Glucos-aminidase			N-Acetate Esterase			N-AS Acet Esterase		
		No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.
KIDNEY	Prox. tubule																		
	Prox. portion	N	S	3+	N	S	4+	N	Sd	40	F	S	1+		d	2+	N	G	2+
	Mid portion	N	S	3+	N	S	3+	N	Sd	40	F	S	1+		d	3+	N	G	2+
	Distal portion	N	S	1+	N	S	3+	N	Sd	40	N	S	4+		d	4+	N	G	4+
	Distal tubule	F	S	1+	F	S	1+		d	±	F	S	1+		d	1+	F	G	±
	Loop of Henle	F	S	1+		d	±	-	-	-	-	-	-	-	-	-	F	G	±
	Collecting tubule																		
	Principal cells	N	S	1+	F	S	1+	-	-	-	-	-	-		-	-	-	-	-
	Intercalated cells	N	S	4+	F	S	4+	N	S	4+	N	S	4+		d	2+	F	G	1+
	Glomerulus	F	S	1+	-	-	-	-	-	-	F	S	3+		d	1+	N	G	1+
	Visc. epith.	F	S	1+	-	-	-	-	-	-	-	-	-	-	-	-	F	G	±
	Parietal epith.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Endothelium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LIVER	Lobule																		
	Central	N		2+	N		1+	F		±	-	-	-		d	4+	N	SG	2+
	Peripheral	N		3+	N		2+	F		1+	F		1+		d	4+	N	SG	2+
	Parenchymal cells																		
	Pericanalicular	N	S	1+	N	S	3+	F	S	1+	-	-	-		d	4+	N	SG	3+
	Perinuclear	-	-	-		d	±	-	-	-	-	-	-		d	4+		Gd	2+
	Kupffer cells	N	S	4+	N	Sd	4+		d	2+	F	Sd	1+		d	4+		Gd	3+
	Bileduct epithelium	F	S	1+	N	S	3+	-	-	-	-	-	-		d	4+		SG	±

EXP. 6 TESTOSTERONE

Table III-15

ANIMAL MOUSE 5	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N S 3+	N S 4+	N Sd 3+	F S 1+	d 2+	N G 2+
Mid portion	N S 2+	N S 3+	N Sd 1+	F S 1+	d 3+	N G 2+
Distal portion	N S 1+	N S 3+	N Sd 1+	N S 2+	d 4+	N G 4+
Distal tubule	F S 1+	F S 1+	d ±	F S ±	d 1+	F G ±
Loop of Henle	F S 1+	d ±	- - -	- - -	- - -	F G ±
Collecting tubule						
Principal cells	F S 1+	F S 1+	- - -	- - -	- - -	- - -
Intercalated cells	N S 4+	F S 4+	d ±	N S 3+	d 2+	F G 1+
Glomerulus	F S 1+	- - -	- - -	F S 3+	d 1+	N G 1+
Visc. epith.	F S 2+	d 1+	- - -	- - -	- - -	F G ±
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 1+	N 1+	F 1+	- - -	d 3+	N 2+
Peripheral	N 2+	N 2+	F 2+	F 1+	MG 4+	N 3+
Parenchymal cells						
Pericanalicular	F S 1+	N S 3+	F 3+	- - -	N Md 4+	N G 3+
Perinuclear	- - -	F Sd ±	- - -	- - -	d 4+	Gd 3+
Kupffer cells	N SM 4+	N Sd 4+	d 1+	d 1+	Gd 4+	Gd 3+
Bileduct epithelium	F S 1+	N S 3+	- - -	- - -	d 4+	N G ±

EXP. 6 TESTOSTERONE

Table III-16

ANIMAL MOUSE 10	ENZYME					
CONTROL TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N SM 3+	N S 4+	N S 4+	F S 1+	d 2+	N G 2+
Mid portion	N S 2+	N S 3+	N S 4+	F S 1+	d 3+	N G 2+
Distal portion	N S 2+	N S 3+	N S 3+	N S 3+	d 4+	N G 4+
Distal tubule	F S 1+	F S 1+	F S 2+	F S ±	d 1+	F G ±
Loop of Henle	F S ±	d ±	- - -	- - -	- - -	F G ±
Collecting tubule						
Principal cells	F S 1+	F S 1+	- - -	- - -	- - -	- - -
Intercalated cells	N S 4+	F S 4+	F S 2+	N S 4+	d 2+	F G 1+
Glomerulus	F S ±	- - -	- - -	F S 4+	d 1+	N G 1+
Visc. epith.	F S 2+	d 1+	- - -	- - -	- - -	F G ±
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 2+	N 1+	F 1+	- - -	d 4+	Gd 2+
Peripheral	N 3+	N 2+	N 3+	F 1+	Gd 4+	Gd 3+
Parenchymal cells						
Pericanalicular	N S 2+	N S 3+	N S 4+	- - -	N Gd 4+	N Gd 3+
Perinuclear	- - -	F Sd ±	- - -	- - -	d 4+	Gd 3+
Kupffer cells	N SM 4+	N Sd 4+	N SM 4+	d 1+	Gd 4+	Gd 3+
Bileduct epithelium	F S 1+	N S 3+	F S 2+	- - -	d 4+	N Gd ±

EXP. 7 ESTRONE

Table III-17

ANIMAL MOUSE 6	ENZYME																	
	APase Gomori			APase Barka			Glucuronidase			Glucosaminidase			N-Acetate Esterase			N-AS Acet Esterase		
TISSUES	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.
KIDNEY Prox. tubule																		
Prox. portion	N	S	3+	N	M	4+	N	SM	4+	F	S	1+		d	3+	N	G	2+
Mid portion	N	S	2+	N	M	4+	N	SM	3+	F	S	1+		d	3+	N	G	2+
Distal portion	N	S	2+	N	M	4+	N	SM	3+	N	S	2+		d	4+	N	G	4+
Distal tubule	F	S	1+	F	S	1+	-	-	-	F	S	1+		d	1+	F	G	±
Loop of Henle	F	S	1+	-	-	-	-	-	-	-	-	-	-	-	-	F	G	±
Collecting tubule																		
Principal cells	F	S	1+	F	S	1+	-	-	-	-	-	-	-	-	-	-	-	-
Intercalated cells	N	S	4+	F	S	4+	F	SM	3+	N	S	4+		d	2+	F	G	1+
Glomerulus	-	-	-	-	-	-	-	-	-	N	S	4+		d	1+	N	G	1+
Visc. epith.	F	S	2+		d	1+	-	-	-	-	-	-	-	-	-	F	G	±
Parietal epith.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Endothelium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LIVER Lobule																		
Central	N		2+	N		2+		d	±	-	-	-		d	4+	N	G	2+
Peripheral	N		2+	N		3+		d	1+	F		1+		d	4+	N	G	3+
Parenchymal cells																		
Pericanalicular	N	S	3+	N	S	3+	F	Sd	±	-	-	-		d	4+	N	G	3+
Perinuclear	-	-	-		d	±	-	-	-	-	-	-		d	4+	N	Gd	3+
Kupffer cells	N	SM	4+	N	Sd	4+	F	Sd	±		d	1+		d	4+	N	Gd	3+
Bileduct epithelium	F	S	1+	N	S	3+	-	-	-	-	-	-		d	4+	N	F	±

Table III-18

EXP. 7 ESTRONE

ANIMAL MOUSE 7	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N S 3+	N M 4+	N SM 4+	F S 1+	d 3+	
Mid portion	N S 2+	N M 4+	N SM 3+	F S 1+	d 3+	
Distal portion	N S 2+	N M 4+	N SM 3+	N S 2+	d 4+	
Distal tubule	F S 1+	F S 1+	- - -	F S 1+	d 1+	
Loop of Henle	F S 1+	- - -	- - -	- - -	- - -	
Collecting tubule						
Principal cells	F S 1+	F S 1+	- - -	- - -	- - -	
Intercalated cells	N S 4+	F S 4+	F SM 2+	N S 4+	d 2+	
Glomerulus	- - -	- - -	- - -	N S 4+	d 1+	
Visc. epith.	F S 2+	d 1+	- - -	- - -	- - -	
Parietal epith.	- - -	- - -	- - -	- - -	- - -	
Endothelium	- - -	- - -	- - -	- - -	- - -	
LIVER Lobule						
Central	N 2+	N 2+	F 1+	- - -	d 4+	
Peripheral	N 3+	N 3+	F 2+	F 1+	d 4+	
Parenchymal cells						
Pericanalicular	N S 3+	N S 3+	F S 1+	- - -	d 4+	
Perinuclear	- - -	d +	- - -	- - -	d 4+	
Kupffer cells	N SM 4+	N Sd 4+	- - -	d 1+	d 4+	
Bileduct epithelium	F S 1+	N S 3+	F S 1+	- - -	d 4+	

EXP. 7 ESTRONE

Table III-19

ANIMAL MOUSE 8	E N Z Y M E																	
TISSUES	APase Gomori			APase Barka			Glucuron-idase			Glucos-aminidase			N-Acetate Esterase			N-AS Acet Esterase		
	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.
KIDNEY Prox. tubule																		
Prox. portion	N	S	4+	N	S	4+	F	Sd	±	F	S	1+		d	4+	N	G	2+
Mid portion	N	S	2+	N	S	4+	-	-	-	F	S	1+		d	3+	N	G	2+
Distal portion	N	S	2+	N	S	3+	-	-	-	N	S	2+		d	4+	N	G	4+
Distal tubule	F	S	1+	F	S	1+	-	-	-	F	S	1+		d	1+	F	G	±
Loop of Henle	F	S	1+	-	-	-	-	-	-	-	-	-	-	-	-	F	G	±
Collecting tubule																		
Principal cells	F	S	1+	F	S	1+	-	-	-	-	-	-	-	-	-	-	-	-
Intercalated cells	N	S	4+	F	S	4+	F	S	1+	N	S	4+		d	2+	F	G	1+
Glomerulus	-	-	-	-	-	-	F	S	2+	N	S	4+		d	1+	N	G	1+
Visc. epith.	F	S	2+		d	1+	-	-	-	-	-	-	-	-	-	F	G	±
Parietal epith.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Endothelium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LIVER Lobule																		
Central	N		2+	N		2+	F		±	-	-	-		d	3+	N	Gd	2+
Peripheral	N		3+	N		3+	F		2+	F		2+		d	4+	N	Gd	3+
Parenchymal cells																		
Pericanalicular	N	S	3+	N	S	3+	F	S	1+	-	-	-		d	4+	N	G	3+
Perinuclear	-	-	-		d	±	-	-	-	-	-	-		d	4+	N	Gd	3+
Kupffer cells	N	SM	4+	N	Sd	4+	-	-	-		d	2+		d	4+	N	Gd	3+
Bileduct epithelium	F	S	1+	N	S	3+	-	-	-	-	-	-		d	4+	M	G	±

EXP. 7 ESTRONE

Table III-20

ANIMAL	MOUSE 9	ENZYME					
	CONTROL	APase Gomori	APase Barka	Glucuron-idase	Glucos-aminidase	N-Acetate Esterase	N-AS Acet Esterase
TISSUES		No.Si.St.	No.Si.St.	No.Si.St.	No.Si.St.	No.Si.St.	No.Si.St.
KIDNEY	Prox. tubule						
	Prox. portion	N S 4+	N S 4+	N S 4+	F S 1+	d 4+	N G 2+
	Mid portion	N S 3+	N S 4+	N S 4+	F S 1+	d 3+	N G 2+
	Distal portion	N S 2+	N S 3+	N S 3+	N S 2+	d 4+	N G 4+
	Distal tubule	F S 1+	F S 1+	N S 2+	F S ±	d 1+	F G ±
	Loop of Henle	F S 1+	- - -	- - -	- - -	- - -	F G ±
	Collecting tubule						
	Principal cells	F S 1+	F S 1+	- - -	- - -	- - -	- - -
	Intercalated cells	N S 4+	F S 4+	F S 2+	N S 4+	d 2+	F G 1+
	Glomerulus	- - -	- - -	- - -	F S 4+	d 1+	N G 1+
	Visc. epith.	F S 2+	d 1+	- - -	- - -	- - -	F G ±
	Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
	Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER	Lobule						
	Central	N 2+	N 2+	F 1+	- - -	d 4+	N Gd 2+
	Peripheral	N 3+	N 3+	F 2+	F 1+	d 4+	N Gd 3+
	Parenchymal cells						
	Pericanalicular	N S 3+	N S 3+	N S 2+	- - -	d 4+	N Gd 3+
	Perinuclear	- - -	d ±	d ±	- - -	d 4+	N Gd 3+
	Kupffer cells	N SM 4+	N Sd 4+	- - -	d 1+	d 4+	N Gd 3+
	Bileduct epithelium	F S 1+	N S 3+	F S 2+	- - -	d 4+	N G ±

Table III-21

ANIMAL RAT I	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N SM 2+	N Sd 2+	F S 2+	F Sd 2+	N Gd 4+	N SG 4+
Mid portion	F SM 2+	N Sd 2+	F S 1+	N S 3+	N Gd 3+	N SG 4+
Distal portion	N S 2+	N Sd 2+	F S ±	N S 2+	N Gd 2+	N SG 3+
Distal tubule	F S ±	F Sd 1+	- - -	F S ±	- - -	- - -
Loop of Henle	F S ±	- - -	- - -	- - -	- - -	F S ±
Collecting tubule						
Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
Intercalated cells	F S ±	F Sd 2+	F S 2+	F S 2+	F Gd 1+	N SG 4+
Glomerulus	- - -	F Sd 1+	N S 3+	N S 2+	F Gd ±	F S 2+
Visc. epith.	F S ±	F Sd 1+	- - -	- - -	- - -	F S ±
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 2+	N 2+	N 2+	- - -	N Gd 2+	N G 3+
Peripheral	N 3+	N 3+	N 3+	F 1+	N Gd 3+	N G 4+
Parenchymal cells						
Pericanalicular	N S 3+	N SM 3+	N Sd 4+	F S 1+	N G 3+	N G 4+
Perinuclear	- - -	- - -	- - -	- - -	N G 3+	N G 2+
Kupffer cells	N S 4+	F SM 4+	N SM 4+	F SM 1+	N G 3+	N G 2+
Bileduct epithelium	F S 1+	- - -	F S 1+	- - -	F G 2+	F G 1+
PANCREAS						
Islet cells	N SG 3+	N Sd 2+	F Sd 1+	N Sd 2+	Gd 2+	N S 3+
Acinar cells	N S 2+	F Sd 2+	- - -	- - -	Gd 2+	N S 4+
Duct epithelium	N S 2+	F S 2+	N S 2+	F S 1+	F Gd 2+	F S 1+

Table III-21

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ANIMAL RAT 1	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N SL 4+	N SMd 4+	F SM 4+	F Sd 2+	F SL 4+	N G 4+
Cords of Billroth	F S 1+	d 1+	F S ±	- - -	- - -	F S 1+
White pulp						
Macrophages	N S 2+	F SMd 4+	F SM 3+	F Sd 2+	d ±	N SM 4+
Lymphoid cells	- - -	d 1+	F S ±	- - -	- - -	F G 1+
DUODENUM						
Villus, epithelium						
Apex	F SM 1+	F Sd 2+	- - -	F S 1+	N Gd 4+	N SG 3+
Middle	N S 2+	F Sd 2+	- - -	N S 2+	N Gd 4+	N SG 2+
Crypt	N S 2+	F Sd 2+	- - -	N S 2+	N Gd 4+	N SG 1+
Lamina propria macroph.	N SL 4+	F SMd 3+	F SM 3+	F S 3+	- - -	F SG 4+
BRAIN						
Neurones Cortical	N S 3+	N Sd 2+	- - -	F S 1+	d 2+	N S 4+
Purkinje	F S 1+	N Sd 2+	- - -	N S 1+	d 2+	F G ±
Glial cells	F S ±	F Sd 1+	- - -	- - -	- - -	F G 1+
Endothelial cells	- - -	- - -	- - -	- - -	d 1+	F G 1+
Perithelial microglia cells	N S 4+	N Sd 3+	N S 4+	N S 4+	F S 4+	N S 4+

Table III-22

ANIMAL RAT 2	E N Z Y M E					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N SM 2+	N Sd 2+	F S 2+	F SM 4+	N Gd 4+	N SG 4+
Mid portion	F SM 2+	N Sd 2+	F SM 1+	N SM 3+	N Gd 2+	N SG 4+
Distal portion	N S 2±	N Sd 2+	F S ±	N S 2+	N Gd 2+	N SG 3+
Distal tubule	F S ±	F Sd ±	- - -	F S ±	- - -	- - -
Loop of Henle	F S ±	- - -	- - -	- - -	- - -	F S ±
Collecting tubule						
Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
Intercalated cells	F S ±	F Sd 1+	F S 2+	F S 2+	F Gd ±	N SG 4+
Glomerulus	- - -	F Sd 1+	N S 3+	N S 2+	F Gd ±	F S 2+
Visc. epith.	F S ±	F Sd ±	- - -	- - -	- - -	- - -
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 2+	N 2+	N 2+	- - -	N G 2+	N G 3+
Peripheral	N 3+	N 3+	N 3+	F 1+	N G 3+	N G 4+
Parenchymal cells						
Pericanalicular	N S 3+	N SM 3+	N Sd 4+	F S 1+	N G 3+	N G 4+
Perinuclear	- - -	- - -	- - -	- - -	N G 3+	N G 2+
Kupffer cells	N SL 4+	F SM 4+	N SM 4+	F SM 1+	N G 3+	N G 2+
Bileduct epithelium	F S 1+	- - -	F S 1+	- - -	F G 2+	F G 1+
PANCREAS						
Islet cells	N SG 3+	N Sd 2+	F Sd 1+	N Sd 2+	Gd 2+	N S 3+
Acinar cells	N S 2+	F Sd 2+	- - -	- - -	Gd 2+	N S 4+
Duct epithelium	N S 2+	F S 2+	N S 2+	F S 1+	F SG 2+	F S 1+

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Table III-22

ANIMAL RAT 2	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N SM 4+	N SMd 4+	F SM 4+	F Sd 2+	F SL 4+	N SG 4+
Cords of Billroth	F S 1+	d 1+	F S ±	- - -	- - -	F S 1+
White pulp						
Macrophages	N S 3+	F Sd 4+	F SM 3+	F Sd 2+	d ±	N SM 4+
Lymphoid cells	- - -	d 1+	F S ±	- - -	- - -	F G 1+
DUODENUM						
Villus, epithelium						
Apex	N SG 2+	F Sd 2+	- - -	F S ±	N Gd 4+	N SG 3+
Middle	N SG 2+	F Sd 2+	- - -	N S 2+	N Gd 4+	N SG 2+
Crypt	N S 2+	F Sd 2+	- - -	N S 2+	N Gd 4+	N SG 1+
Lamina propria macroph.	N SM 4+	F SMd 3+	F SM 3+	F S 3+	- - -	F SG 4+
BRAIN						
Neurones Cortical	N S 3+	N Sd 2+	- - -	F S 1+	d 2+	N S 4+
Purkinje	F S 1+	N Sd 2+	- - -	F S 1+	d 2+	F G ±
Glial cells	F S ±	F Sd 1+	- - -	- - -	- - -	F G 1+
Endothelial cells	- - -	- - -	- - -	- - -	d 1+	F G 1+
Perithelial microglia cells	N S 4+	N Sd 3+	N S 4+	N S 4+	F S 4+	N S 4+

Table III-23

ANIMAL RAT 3	E N Z Y M E					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N SL 2+	N SM 3+	F SM 2+	F Sd 4+	N Gd 3+	N SG 4+
Mid portion	N SM 2+	N SM 3+	F SM 2+	N SM 4+	N Gd 2+	N SG 4+
Distal portion	N S 2+	N SM 3+	F S ±	N S 2+	N Gd 2+	N SG 3+
Distal tubule	F S ±	F Sd 1+	F S ±	F S ±	- - -	- - -
Loop of Henle	F S ±	- - -	F S ±	- - -	- - -	F S ±
Collecting tubule						
Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
Intercalated cells	F S 1+	F Sd 2+	N SM 4+	F S 2+	F G 1+	N S 4+
Glomerulus	- - -	F Sd 1+	F S ±	N S 2+	F G ±	F S 2+
Visc. epith.	F S ±	F Sd 1+	F S 1+	- - -	- - -	F S ±
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 1+	N 2+	N 2+	- - -	N G 2+	N G 3+
Peripheral	N 2+	N 3+	N 3+	F 1+	N G 3+	N G 4+
Parenchymal cells						
Pericanalicular	N S 3+	N SM 3+	N Sd 4+	F S 1+	N G 3+	N SG 4+
Perinuclear	- - -	- - -	- - -	- - -	N G 3+	N G 2+
Kupffer cells	N SL 4+	N SL 4+	N SM 4+	F SM 1+	N G 3+	N G 2+
Bileduct epithelium	F S 1+	- - -	F S 1+	- - -	F G 2+	F G 1+
PANCREAS						
Islet cells	N SG 3+	N Sd 2+	F Sd 1+	N Sd 2+	Gd 2+	N S 3+
Acinar cells	N S 2+	F Sd 3+	- - -	- - -	Gd 2+	N S 4+
Duct epithelium	N S 2+	F S 2+	N S 2+	F S 1+	F Sd 2+	F S 1+

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Table III-23

ANIMAL RAT 3	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N SL 4+	N SMd 4+	F SM 4+	F Sd 2+	F SL 4+	N SG 4+
Cords of Billroth	F S ±	d 2+	F S 1+	- - -	- - -	F S 1+
White pulp						
Macrophages	N S 4+	F SMd 4+	F SM 4+	F Sd 2+	d ±	N SM 4+
Lymphoid cells	F S ±	d 2+	F S 1+	- - -	- - -	F G 1+
DUODENUM						
Villus, epithelium						
Apex	F Sd 1+	F Sd 2+	- - -	F S ±	N Gd 4+	N SG 3+
Middle	N S 2+	F Sd 2+	- - -	N S 2+	N Gd 4+	N SG 2+
Crypt	N SG 2+	F Sd 2+	- - -	N S 2+	N Gd 4+	N SG 1+
Lamina propria macroph.	N SG 4+	F SMd 3+	F SM 3+	F S 3+	- - -	F SG 4+
BRAIN						
Neurones Cortical	N S 3+	N Sd 2+	- - -	F Sd 1+	d 2+	N SG 4+
Purkinje	F S 1+	N Sd 2+	- - -	F Sd 1+	d 2+	F G ±
Glial cells	F S ±	F Sd 1+	- - -	- - -	- - -	F G 1+
Endothelial cells	- - -	- - -	- - -	- - -	F Sd 1+	F G 1+
Perithelial microglia cells	N S 4+	N Sd 4+	N SM 4+	N S 4+	F S 4+	N S 4+

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Table III-24

ANIMAL RAT 4	ENZYME					
TISSUES	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
KIDNEY Prox. tubule						
Prox. portion	N SL 4+	N SM 4+	F M 3+	F SMd 2+	d 4+	N SG 4+
Mid portion	N SM 4+	N SM 4+	F SM 2+	F S 2+	d 4+	N SG 4+
Distal portion	N S 4+	N SM 4+	F S 1+	F S 1+	d 4+	N SG 3+
Distal tubule	F S 1+	F S 2+	F S ±	- - -	- - -	- - -
Loop of Henle	F S ±	F S ±	F S ±	- - -	- - -	F S ±
Collecting tubule						
Principal cells	- - -	- - -	- - -	- - -	- - -	- - -
Intercalated cells	N S 2+	N SM 3+	N SM 4+	F S 1+	F SG 2+	N SG 4+
Glomerulus	F S 1+	F S 2+	F S ±	F S 1+	F SG 1+	F S 2+
Visc. epith.	F S 3+	F S 1+	F S 1+	- - -	- - -	F S ±
Parietal epith.	- - -	- - -	- - -	- - -	- - -	- - -
Endothelium	- - -	- - -	- - -	- - -	- - -	- - -
LIVER Lobule						
Central	N 3+	N 3+	N 2+	- - -	N Gd 4+	N G 3+
Peripheral	N 4+	N 4+	N 3+	- - -	N Gd 4+	N G 4+
Parenchymal cells						
Pericanalicular	N Sd 4+	N S 4+	N S 3+	- - -	N Gd 4+	N G 4+
Perinuclear	- - -	- - -	- - -	- - -	N Gd 4+	N G 2+
Kupffer cells	N SMd 4+	F SM 4+	N SM 4+	F SM ±	N Gd 4+	N G 2+
Bileduct epithelium	F S 1+	- - -	F S 1+	- - -	F Gd 2+	F G 1+
PANCREAS						
Islet cells	N SGd 4+	N Sd 3+	F Sd 2+	N S 2+	Gd 2+	N S 3+
Acinar cells	N Sd 3+	F Sd 2+	- - -	- - -	Gd 3+	N S 4+
Duct epithelium	N S 2+	F S 2+	N S 2+	F S 1+	F Gd 2+	F SG 1+

Table III-24

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ANIMAL RAT 4	E N Z Y M E					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N SL 4+	N SM 4+	F SL 4+	F Sd 1+	N SL 4+	N SG 4+
Cords of Billroth	F S 2+	- - -	F S 2+	- - -	d 2+	F S 1+
White pulp						
Macrophages	N SM 4+	F SM 4+	F SL 4+	F Sd 2+	F SL 4+	N SM 4+
Lymphoid cells	F S 1+	- - -	F S 2+	- - -	- - -	F G 1+
DUODENUM						
Villus, epithelium						
Apex	N S 4+	F Sd 3+	F Sd ±	F S ±	N Gd 4+	N SG 3+
Middle	N S 4+	F Sd 3+	F Sd ±	N S 1+	N Gd 4+	N SG 2+
Crypt	N S 4+	F Sd 4+	F Sd ±	N S 1+	N Gd 4+	N SG 1+
Lamina propria macroph.	N S 4+	F SM 4+	N SM 4+	F S 3+	F S 1+	F SG 4+
BRAIN						
Neurones Cortical	N S 4+	N Sd 4+	- - -	F S ±	d 2+	N Sd 4+
Purkinje	F S 2+	N S 3+	- - -	F Sd ±	d 2+	F G ±
Glial cells	F S ±	F S 2+	- - -	- - -	- - -	F G 1+
Endothelial cells	- - -	- - -	- - -	- - -	d 1+	F G 1+
Perithelial microglia cells	N S 4+	N S 4+	N SM 4+	F SM 3+	F S 4+	N S 4+

EXP. IX DEXTRAN

Table III-25

ANIMAL Mouse I	ENZYME																	
TISSUES	APase Gomori			APase Barka			Glucuronidase			Glucosaminidase			N-Acetate Esterase			N-AS Acet Esterase		
	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.
KIDNEY Prox. tubule																		
Prox. portion	N	SM	4+	N	S	4+	N	Sd	4+	F	S	4+		d	4+	N	Gd	4+
Mid portion	N	S	3+	N	S	3+	N	S	4+	F	S	4+		d	4+	N	Gd	4+
Distal portion	F	S	2+	F	S	1+	N	S	4+	F	S	4+		d	4+	N	Gd	4+
Distal tubule	F	S	1+	N	S	2+	F	S	±	F	S	±		d	2+	F	G	1+
Loop of Henle	F	S	2+	F	S	±	-	-	-	-	-	-	-	-	-	F	G	1+
Collecting tubule																		
Principal cells	F	S	1+	-	-	-	-	-	-	-	-	-		d	1+	F	G	1+
Intercalated cells	N	S	2+	N	S	4+	N	S	4+	N	S	4+		d	1+	N	SM	4+
Glomerulus																		
Visc. epith.		d	3+	F	S	±	-	-	-	-	-	-	-	-	-	-	-	-
Parietal epith.		d	3+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Endothelium		d	3+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LIVER Lobule																		
Central	F		1+	F		2+	F		1+	-		-		d	3+	N	Gd	3+
Peripheral	N		3+	N		3+	N		3+	-		-		d	4+	N	Gd	4+
Parenchymal cells																		
Pericanalicular	N	S	3+	F	S	2+	N	S	2+	-	-	-		d	3+	N	Gd	3+
Perinuclear	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	Gd	3+
Kupffer cells	N	LS	4+	N	S	4+	N	LS	4+	F	S	3+		d	3+	N	Gd	3+
Bileduct epithelium	F	S	1+	F	S	1+	-	-	-	-	-	-	-	-	-	N	G	1+
PANCREAS																		
Islet cells	F	L	3+	F	S	3+	F	S	3+	F	S	1+	F	SGd	±	F	G	1+
Acinar cells	F	S	2+	F	S	2+	-	-	-	-	-	-		d	4+	N	G	4+
Duct epithelium	F	S	1+	F	S	3+	F	S	1+	-	-	-		d	4+	N	G	3+

Table III-25

EXP. IX DEXTRAN

ANIMAL Mouse I	ENZYME					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuronidase No.Si.St.	Glucosaminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N S 4+	N SL 4+	N SL 4+	F SM 3+	N SM 4+	N SM 4+
Cords of Billroth	F S	F S 1+	- - -	- - -	- - -	N Gd 3+
White pulp						
Macrophages	N S 4+	N S 4+	N SL 4+	F S 3+	F S	N Gd 3+
Lymphoid cells	F S	F Sd 1+	- - -	- - -	- - -	N Gd 3+
DUODENUM						
Villus, epithelium						
Apex	F S 1+	N S 3+	F Sd 1+	F S	N Gd 4+	N Gd 4+
Middle	F S 1+	N S 3+	F Sd 1+	F S 3+	N Gd 4+	N Gd 4+
Crypt	F S 1+	N S 3+	F Sd 1+	F S 3+	N Gd 4+	N Gd 4+
Lamina propria macroph.	N S 4+	N S 4+	N SL 4+	F S	F G 4+	N GD 4+
BRAIN						
Neurones Cortical	N S 4+	N S 4+	F S 1+	F S 2+	F Gd 1+	N G 2+
Purkinje	N S 4+	N S 4+	F S 1+	F SM 4+	F Gd 1+	N G 3+
Glial cells	N S 2+	N S 2+	F S	- - -	- - -	- - -
Endothelial cells	F S 1+	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	N S 3+	F S 3+	N SL 4+	N SM 4+	F S 4+	N G 4+

Table III-26

EXP. IX DEXTRAN

ANIMAL Mouse II	ENZYME																	
	APase Gomori			APase Barka			Glucuronidase			Glucosaminidase			N-Acetate Esterase			N-AS Acet Esterase		
TISSUES	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.
KIDNEY Prox. tubule																		
Prox. portion	N	SL	4+	N	S	4+	F	S	2+	N	S	4+		d	4+	N	Gd	4+
Mid portion	N	S	4+	N	S	3+	F	S	1+	F	S	3+		d	4+	N	Gd	4+
Distal portion	N	S	3+	N	S	2+	F	S	1+	F	S	3+		d	4+	N	Gd	3+
Distal tubule	N	S	2+	N	S	2+	-	-	-	-	-	-		d	2+	N	Gd	1+
Loop of Henle	F	S	4+	F	S	2+	-	-	-	-	-	-	F	G	1+	F	Gd	1+
Collecting tubule																		
Principal cells	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Intercalated cells	N	S	4+	N	S	4+	N	S	4+	N	S	4+		d	3+	N	S	4+
Glomerulus																		
Visc. epith.		d	3+	F	S	2+	-	-	-	-	-	-	-	-	-	-	-	-
Parietal epith.		d	3+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Endothelium		d	3+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LIVER Lobule																		
Central	N		3+	N		3+	F		2+	-		-		d	3+	N	Gd	3+
Peripheral	N		4+	N		2+	F		3+	-		-		d	4+	N	Gd	4+
Parenchymal cells																		
Pericanalicular	N	S	3+	N	S	3+	F	S	4+	vF	S	1+	N	Gd	3+	N	Gd	4+
Perinuclear	-	-	-	-	-	-	-	-	-	-	-	-	N	Gd	3+	N	Gd	3+
Kupffer cells	N	SM	4+	N	S	4+	F	S	4+	N	S	2+	N	Gd	3+	N	Gd	4+
Bileduct epithelium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PANCREAS																		
Islet cells				N	Sd	2+	F	S	1+				F	G	±	F	G	1+
Acinar cells	F	S	3+	vF	S	±	-	-	-	-	-	-	N	Gd	2+	N	Gd	4+
Duct epithelium	F	S	3+	F	S	1+	-	-	-	-	-	-		d	4+	N	Gd	4+

Table III-26

EXP. IX DEXTRAN

ANIMAL Mouse II	E N Z Y M E					
TISSUE	APase Gomori No.Si.St.	APase Barka No.Si.St.	Glucuron-idase No.Si.St.	Glucos-aminidase No.Si.St.	N-Acetate Esterase No.Si.St.	N-AS Acet Esterase No.Si.St.
SPLEEN						
Red pulp						
Macrophages	N S 4+	N Sd 4+	F SM 4+	N S 3+	N Gd 3+	N Gd 3+
Cords of Billroth	F S 1+	- - -	- - -	- - -	- - -	F G 1+
White pulp						
Macrophages	N SL 4+	F S 4+	F SM 4+	N SL 4+	N Gd 3+	N Gd 3+
Lymphoid cells	F S 1+	- - -	- - -	- - -	- - -	F Gd 1+
DUODENUM						
Villus,epithelium						
Apex	F S 3+	F S 2+	F S 4+	N SM 3+	N Gd 4+	N Gd 4+
Middle	F S 3+	N SM 3+	F S 4+	N SM 3+	N Gd 4+	N Gd 4+
Crypt	F S 3+	N SM 3+	F S 4+	N SM 3+	N Gd 4+	N Gd 4+
Lamina propria macroph.	F SL 4+	N SM 4+	N S 4+	F S 2+	F G 1+	F G 1+
BRAIN						
Neurones Cortical	N S 4+	N S 4+	- - -	N S 3+	N Gd 3+	N G 3+
Purkinje	N S 4+	N SM 4+	F S 2+	N S 3+	N Gd 2+	N G 2+
Glial cells	F S 3+	F S 3+	- - -	F S 2+	F Gd 1+	F Gd 1+
Endothelial cells	F S 1+	- - -	- - -	- - -	- - -	- - -
Perithelial microglia cells	F S 4+	N S 4+	F S 4+	F S 4+	N Gd 4+	N Gd 4+

EXP. IX DEXTRAN

Table III-27

ANIMAL Mouse III	ENZYME																	
	APase Gomori			APase Barka			Glucuronidase			Glucosaminidase			N-Acetate Esterase			N-AS Acet Esterase		
TISSUES	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.
KIDNEY Prox. tubule																		
Prox. portion	N	S	3+	N	S	4+	N	S	2+	N	S	2+		d	2+	N	G	3+
Mid portion	N	S	2+	N	S	4+	N	S	2+	N	S	3+		d	3+	N	G	2+
Distal portion	N	S	2+	N	S	3+	N	S	1+	N	S	3+		d	4+	N	G	2+
Distal tubule	F	S	1+	N	S	1+	-	-	-	-	-	-	-	-	-	-	-	-
Loop of Henle	F	S	1+	N	S	1+	-	-	-	-	-	-	-	-	-	-	-	-
Collecting tubule																		
Principal cells	-	-	-	-	-	-	-	-	-	-	-	-	N	Gd	2+	-	-	-
Intercalated cells	N	SM	4+	N	S	4+	N	S	4+	N	S	4+	N	Gd	4+	N	S	4+
Glomerulus																		
Visc. epith.	F	S	1+	F	S	1+	-	-	-	-	-	-	-	-	-	N	G	±
Parietal epith.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Endothelium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LIVER Lobule																		
Central	N		3+	N		3+	N		2+	-		-	N		4+	N	G	3+
Peripheral	N		3+	N		3+	N		2+	-		-	N		4+	N	G	3+
Parenchymal cells																		
Pericanalicular	N	S	2+	N	S	4+	F	S	3+	vF	S	1+	N	G	3+	N	G	3+
Perinuclear	-	-	-	-	-	-	-	-	-	-	-	-	N	G	3+	N	G	3+
Kupffer cells	N	S	4+	N	S	4+	F	S	4+	N	S	4+	N	G	4+	N	G	3+
Bileduct epithelium	-	-	-	-	-	-	-	-	-	-	-	-	N	G	4+	N	G	3+
PANCREAS																		
Islet cells	F	S	3+		d	1+	vF	S	±	vF	S	±	-	-	-	F	G	3+
Acinar cells	F	S	1+	F	S	1+	-	-	-	-	-	-	N	Gd	2+	N	SG	4+
Duct epithelium	-	-	-	F	S	3+	F	S	3+	N	S	3+	N	Gd	4+		d	3+

Table III-27

EXP. IX DEXTRAN

ANIMAL Mouse III	ENZYME																	
	APase Gomori			APase Barka			Glucuronidase			Glucosaminidase			N-Acetate Esterase			N-AS Acet Esterase		
TISSUE	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.	No.	Si.	St.
SPLEEN																		
Red pulp																		
Macrophages	N	SL	4+	N	Sd	4+	F	Sd	4+	N	S	3+	F	G	1+	N	SG	4+
Cords of Billroth	–	–	–	–	–	–	F	S	3+	–	–	–	–	–	–	–	–	–
White pulp																		
Macrophages	N	SL	4+	N	Sd	4+	F	Sd	3+	N	Sd	4+	F	G	4+	F	SG	3+
Lymphoid cells	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
DUODENUM																		
Villus, epithelium																		
Apex	N	S	2+	N	Sd	4+	F	Sd	2+	F	SM	4+	N	Gd	4+	N	Gd	4+
Middle	N	S	2+	N	Sd	4+	F	Sd	2+	N	S	4+	N	Gd	4+	N	Gd	4+
Crypt	N	S	2+	N	Sd	4+	F	Sd	2+	N	S	4+	N	Gd	4+	N	Gd	4+
Lamina propria macroph.	N	S	4+	N	SL	4+	N	S	4+	N	S	4+	–	–	–	–	–	–
BRAIN																		
Neurones Cortical	N	S	4+	N	S	4+	F	Sd	±	N	S	2+	–	–	–	–	–	–
Purkinje	N	S	4+	N	S	4+	F	Sd	±	N	S	4+	–	–	–	–	–	–
Glial cells	F	S	1+	F	S	2+	F	Sd	±	N	Sd	2+	–	–	–	F	Sd	3+
Endothelial cells	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Perithelial microglia cells	F	S	4+	N	S	4+	F	S	4+	N	Sd	4+		d	4+	N	SG	3+

Photomicrographs of Histochemical Localization of Lysosomal Acid Hydrolases in Tissues of Vertebrate Species.

Fig. 1. to Fig. 157. inclusive, and

Fig. 159. to Fig. 182. inclusive.

LIST OF ABBREVIATIONS AND SYMBOLS

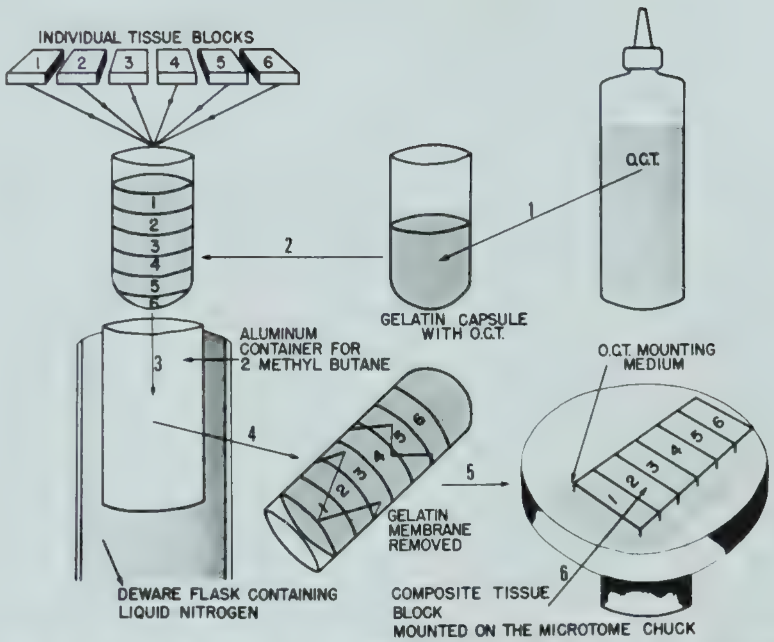
A	Acinus
D	Distal convoluted tubule of kidney
Du	Pancreatic duct
I	Islet of Langerhans
Int	Intercalated cell
K	Kupffer cell
L	Lumen
Lp	Lamina propria
Ly	Lysosomes
Mu	Mucosal epithelium
N	Nucleus
Ne	Neuron
P	Proximal convoluted tubule
Pb	Pigment body
Pm	Perithelial microglia
rbc	Red blood cells
Res	Reticuloendothelial cells
Rp	Red pulp
Wp	White pulp

Fig. 1. Graphic illustration of tissue processing technique employed in this study to obtain sections of six tissues simultaneously.

Fig. 2. Composite 8 micron section of guinea pig tissues processed as shown in Fig. 1. , mounted on a gelatinized coverslip and stained for B-glycerophosphate phosphatase. 4 X.

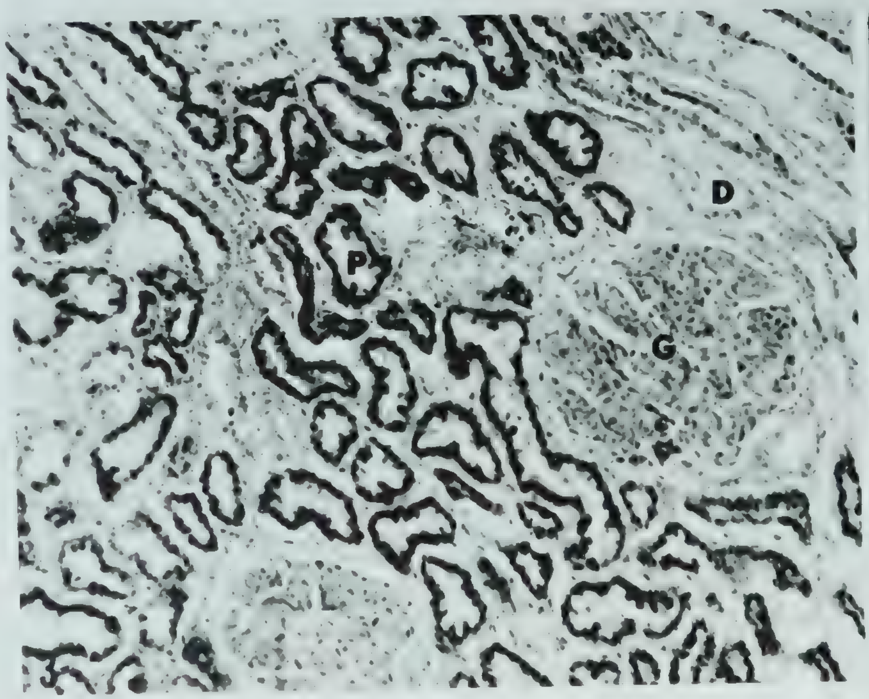
Fig. 3. Photomicrograph of guinea pig kidney taken from section shown in Fig. 2. 250 X.

Fig. 4. Photomicrograph of guinea pig liver taken from section shown in Fig. 2. 250 X.


INDIVIDUAL TISSUE BLOCKS
1
2
3
4
5
6
O.C.T.
1
2
GELATIN CAPSULE WITH O.C.T.
3
ALUMINUM CONTAINER FOR 2 METHYL BUTANE
4
GELATIN MEMBRANE REMOVED
5
O.C.T. MOUNTING MEDIUM
6
DEWARE FLASK CONTAINING LIQUID NITROGEN
COMPOSITE TISSUE BLOCK MOUNTED ON THE MICROTOME CHUCK

1

2


D
P
G

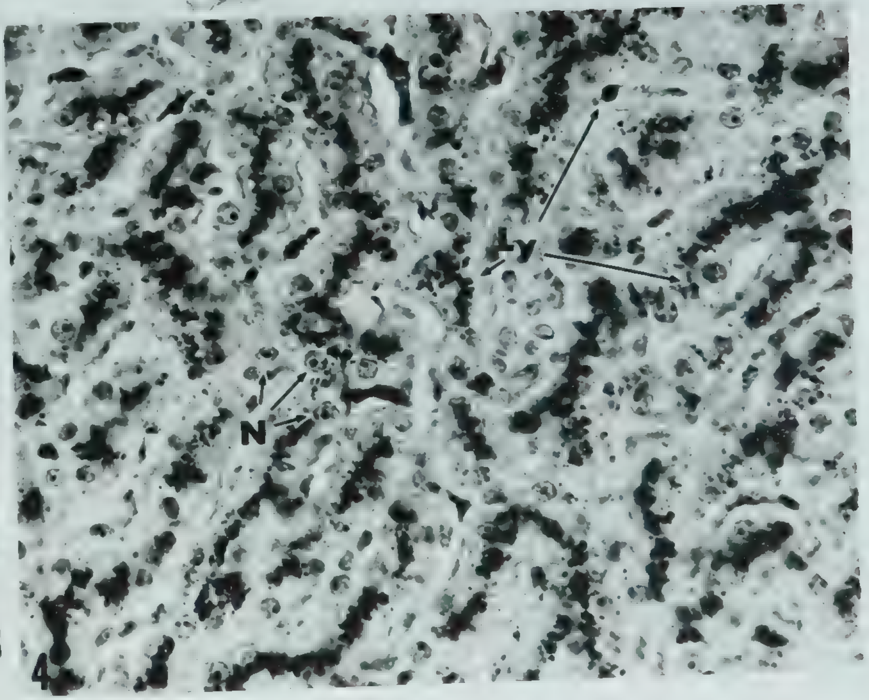
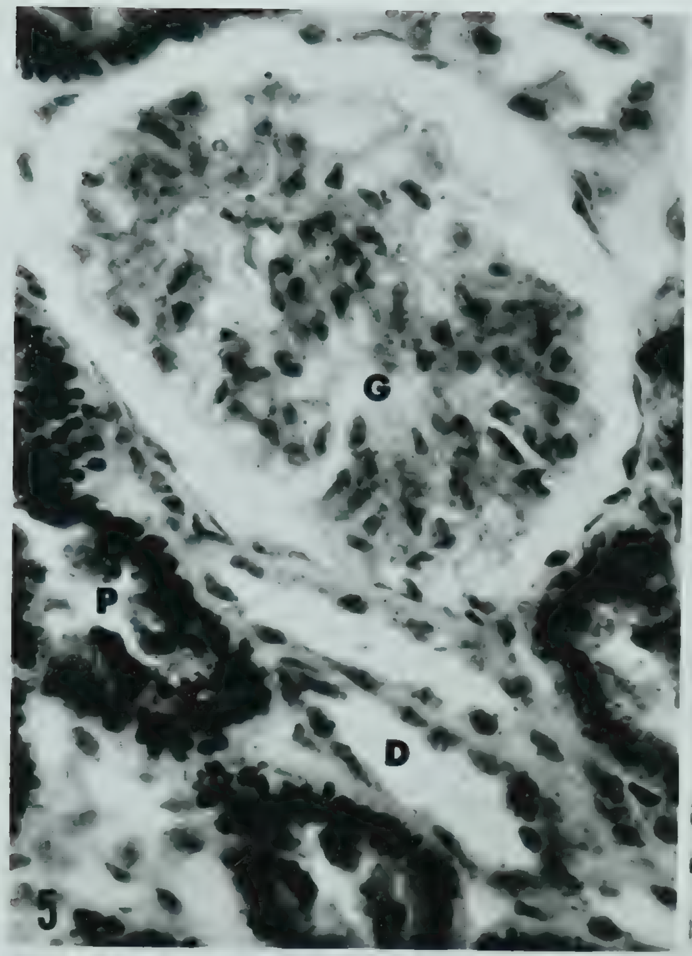

Ly
N
4

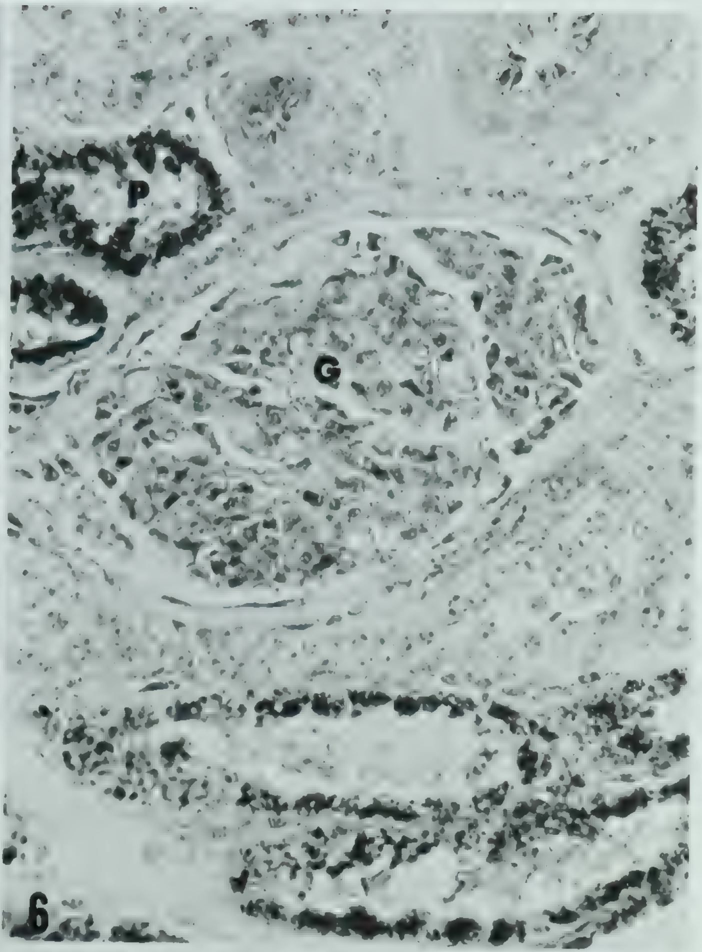
Fig. 5. Rat kidney, B-glycerophosphate phosphatase, note high enzyme activity in proximal convoluted tubules (P), sparse activity in distal convoluted tubule (D). 400 X.

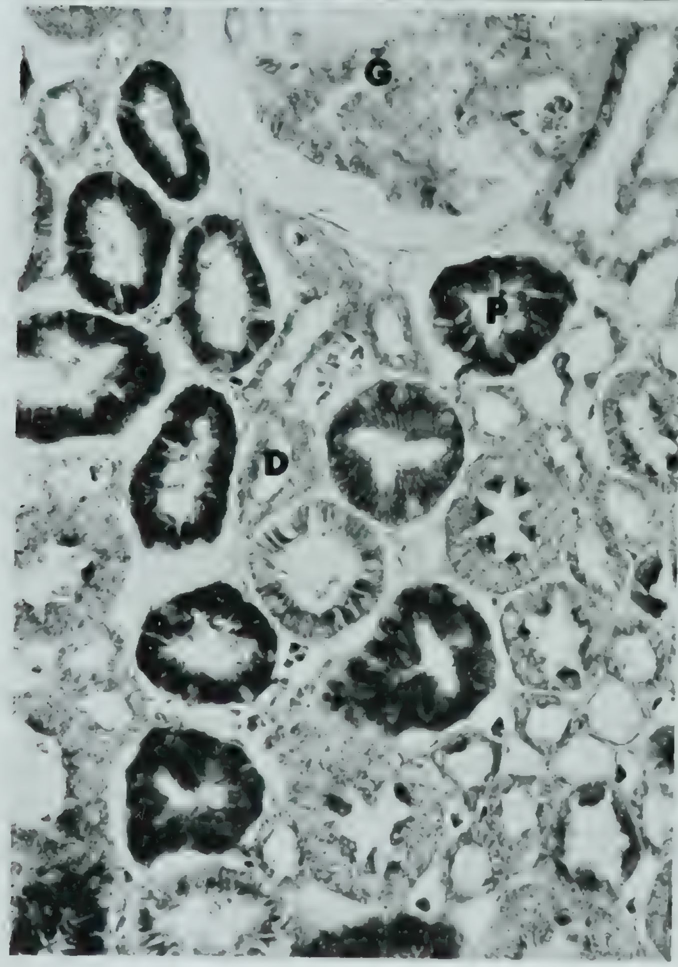
Fig. 6. Horse kidney, B-glycerophosphate phosphatase. 250 X.

Fig. 7. Dog kidney, Naphthol AS-TR phosphate phosphatase, note the high activity of this enzyme in the proximal tubules (P). 250 X.

Fig. 8. Hamster kidney, B-glycerophosphate phosphatase. 250 X.


G
P
D
5


P
G
6


G
P
D

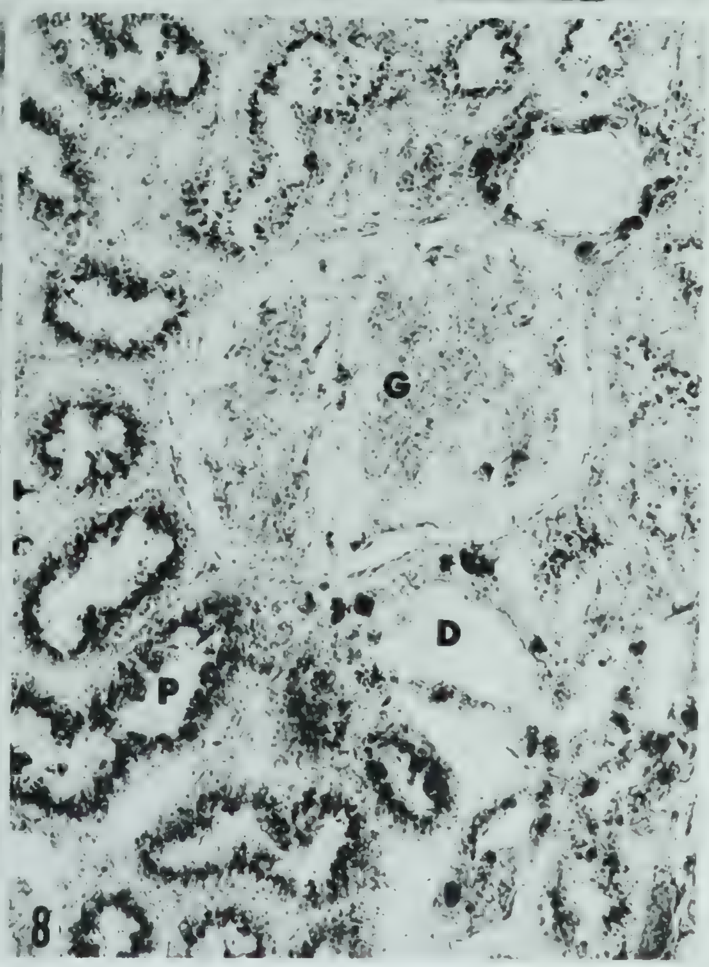
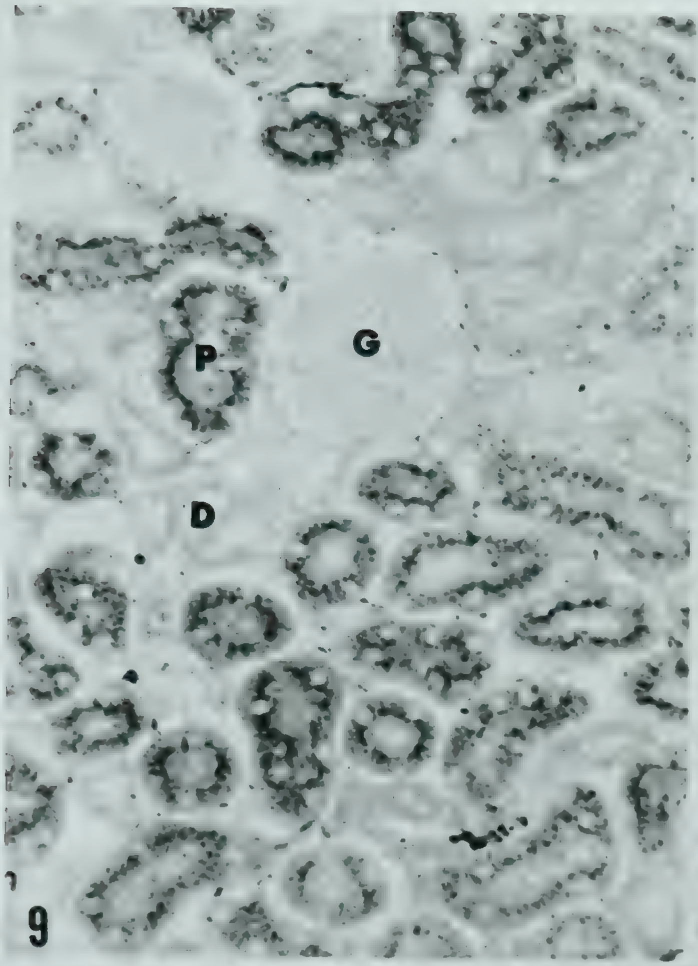

G
D
P
8

Fig. 9. Field mouse kidney, Naphthol AS-TR phosphate phosphatase. 100 X.

Fig. 10. Horse kidney, Naphthol AS-TR phosphate phosphatase, note the large glomerulus (G), and the intense enzyme activity in proximal convoluted tubules (P). 250 X.

Fig. 11. Hamster kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 12. Pig kidney, Naphthol AS-TR phosphate phosphatase. 250 X.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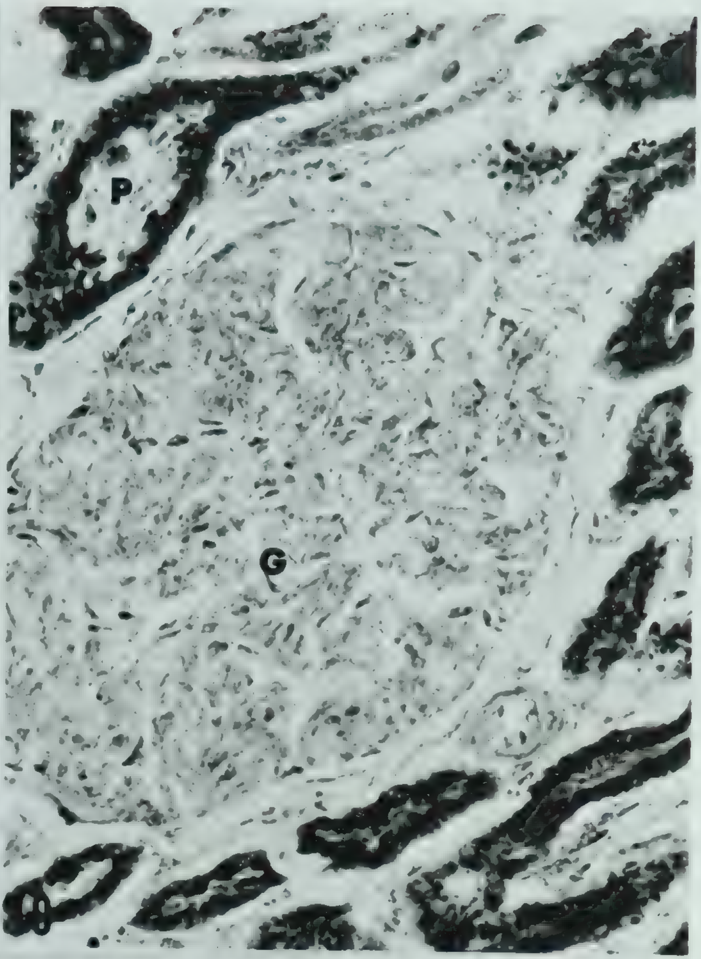
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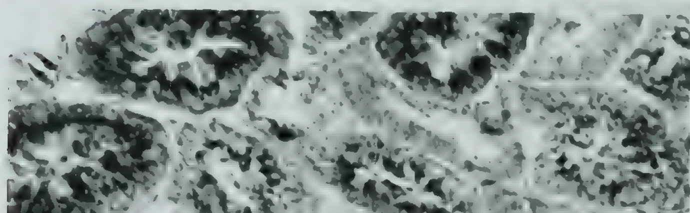
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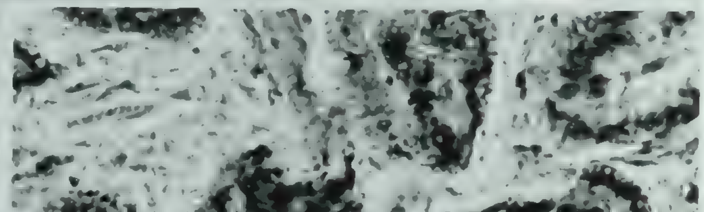
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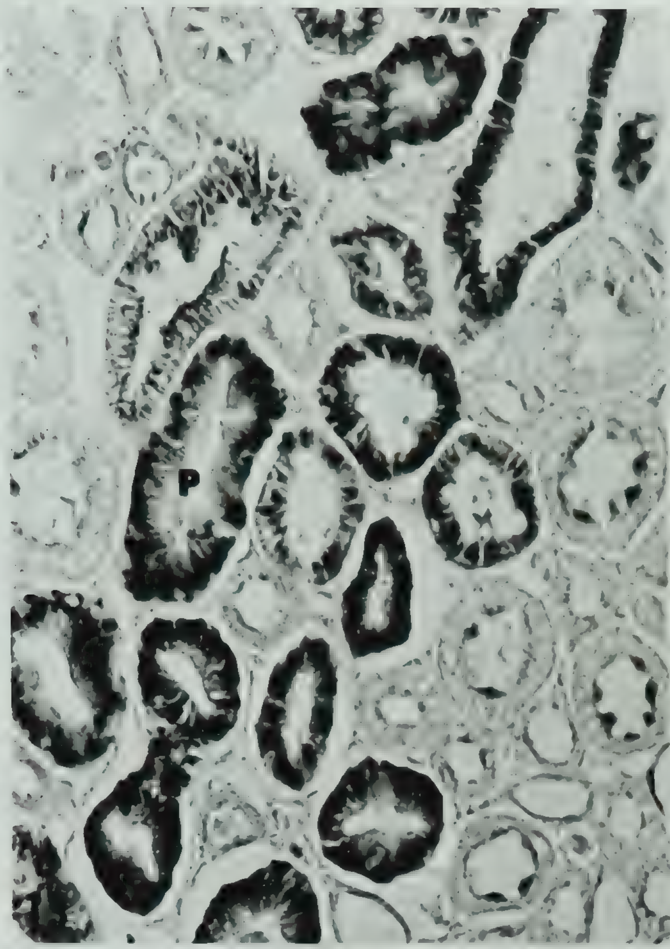
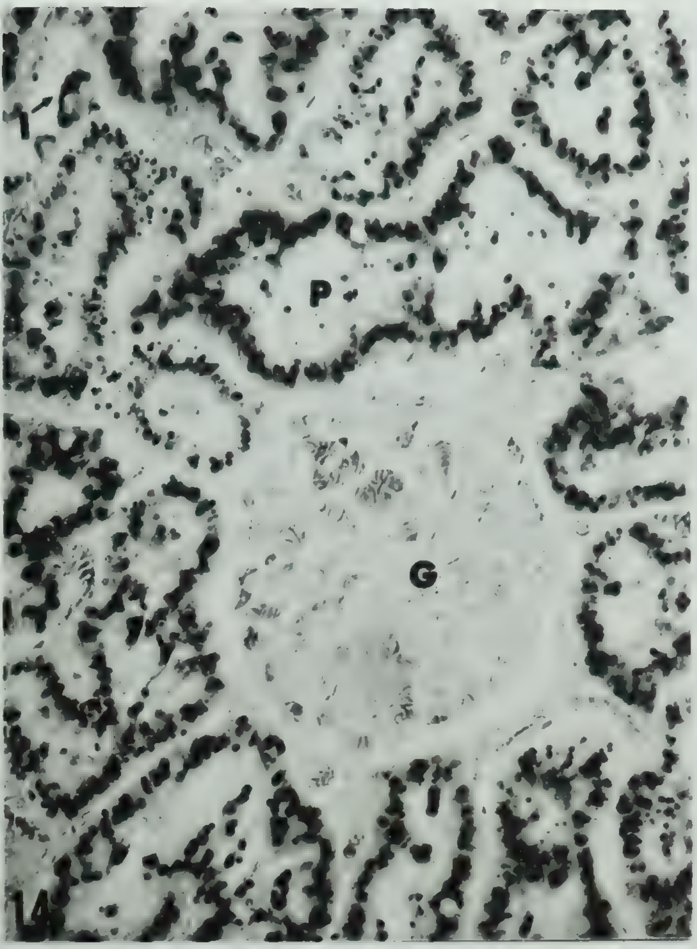
Fig. 13. Dog kidney, B-glucuronidase, note high enzyme activity in lysosomes of the proximal convoluted tubules (P). 250 X.

Fig. 14. Rat kidney, glucosaminidase, note high enzyme activity in collecting tubule intercalated cell (Int), proximal convoluted tubules (P), and the absence of enzyme activity from cells of the glomerulus (G). 250 X.

Fig. 15. Guinea pig kidney, glucosaminidase. 250 X.

Fig. 16. Sheep kidney, glucosaminidase, note the large glomerulus (G). 250 X





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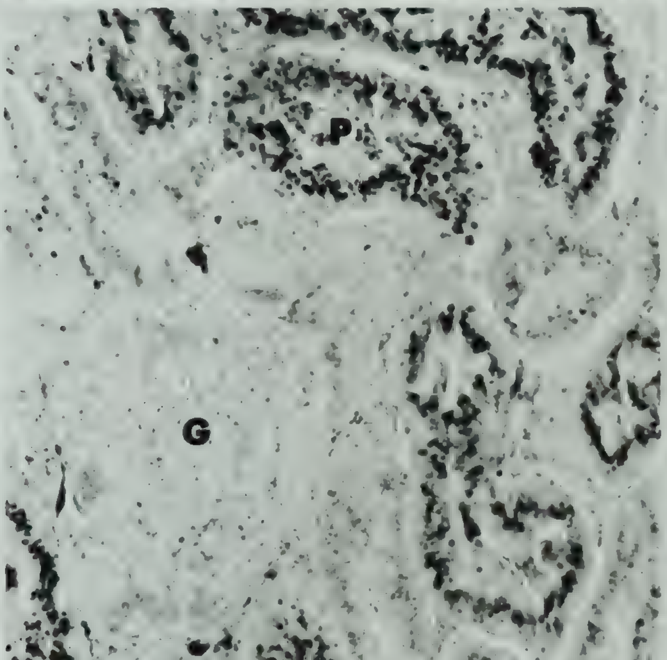

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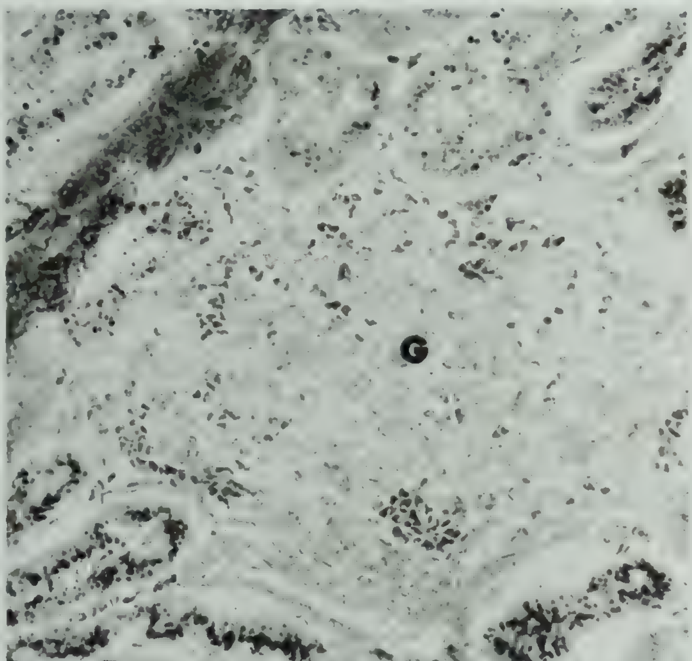

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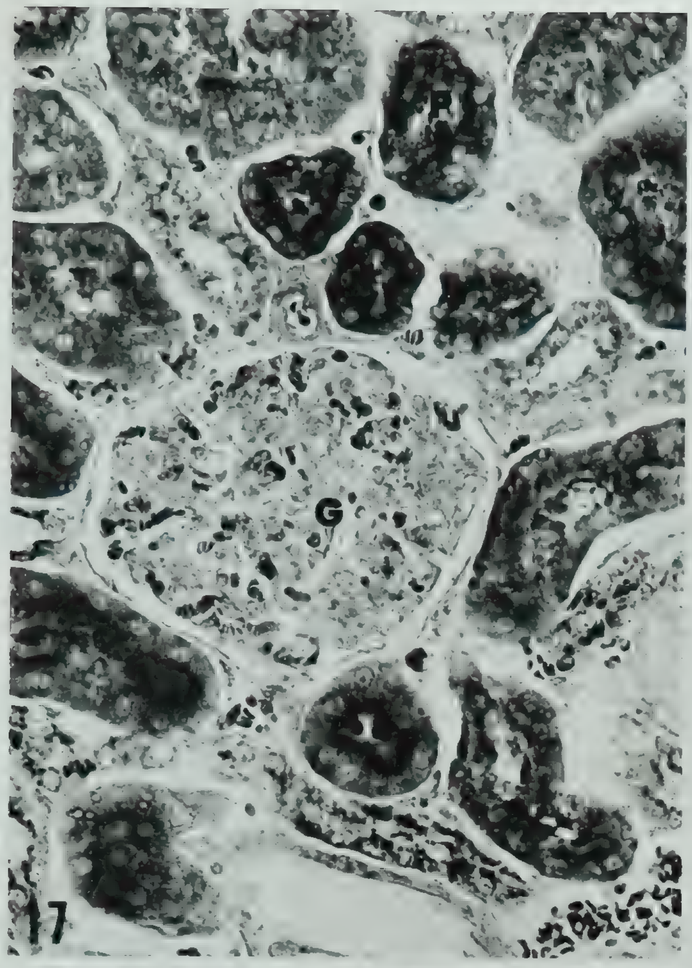
Fig. 17. Cat kidney, glucosaminidase, note the diffuse enzyme activity in the proximal convoluted tubules (P). 250 X.

Fig. 18. Rat kidney, a-Naphthyl acetate esterase, note the high enzyme activity only found in the proximal convoluted tubules (P). 250 X.

Fig. 19. Horse kidney, Naphthol-AS acetate esterase, note the high enzyme activity in the proximal convoluted tubules (P). 250 X.

Fig. 20. Cat kidney, Naphthol-AS acetate esterase, note the high enzyme activity in proximal convoluted tubules (P). 250 X.



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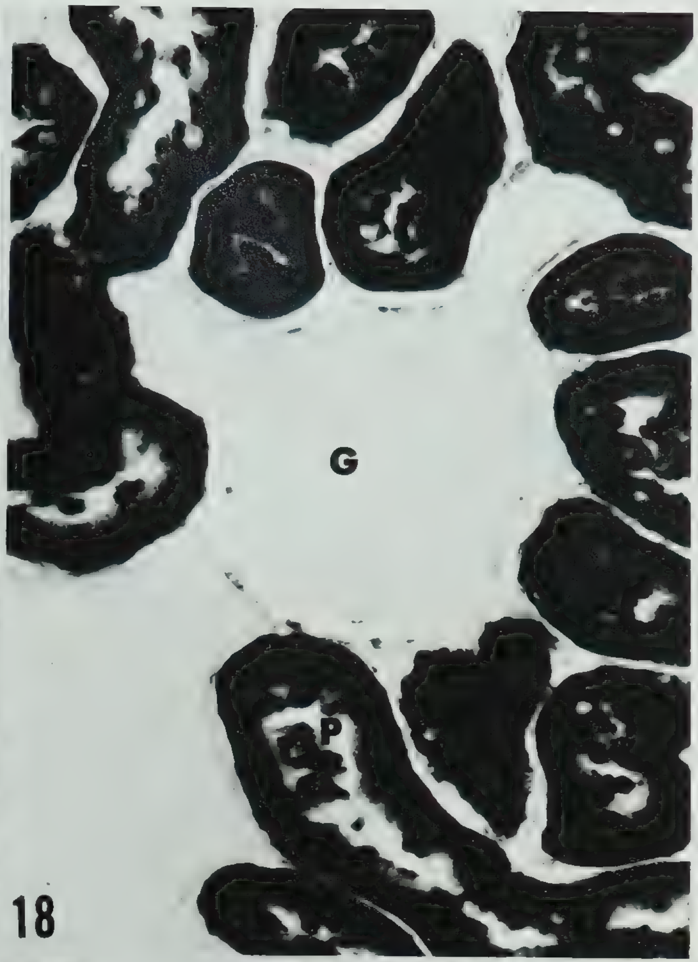

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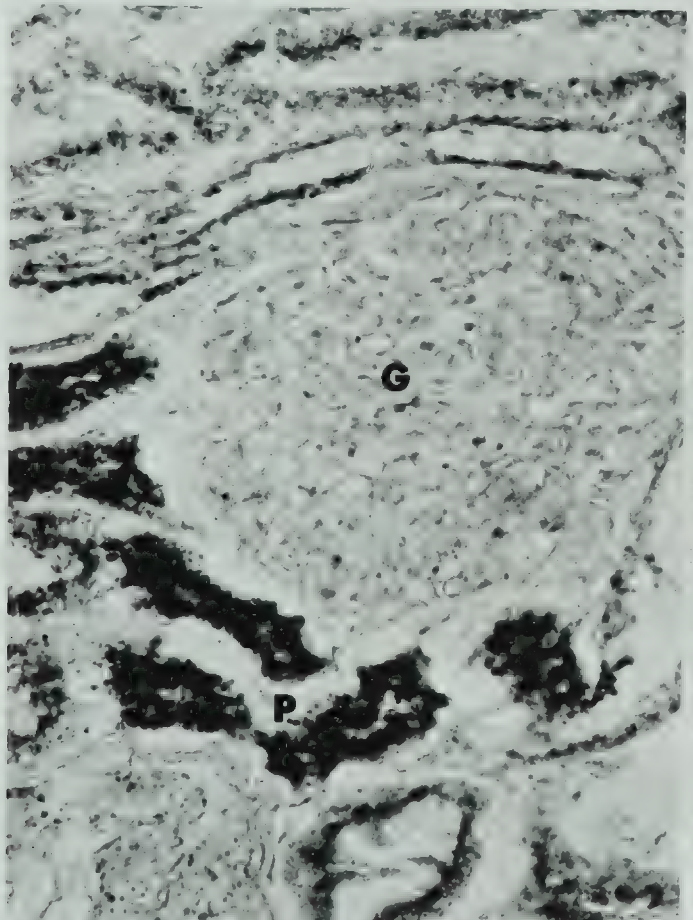

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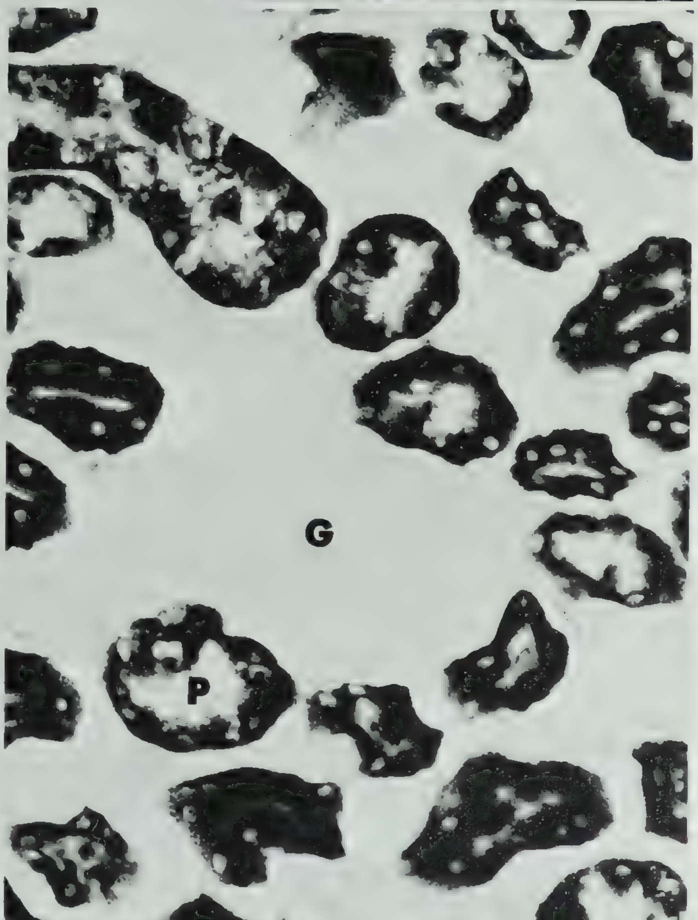
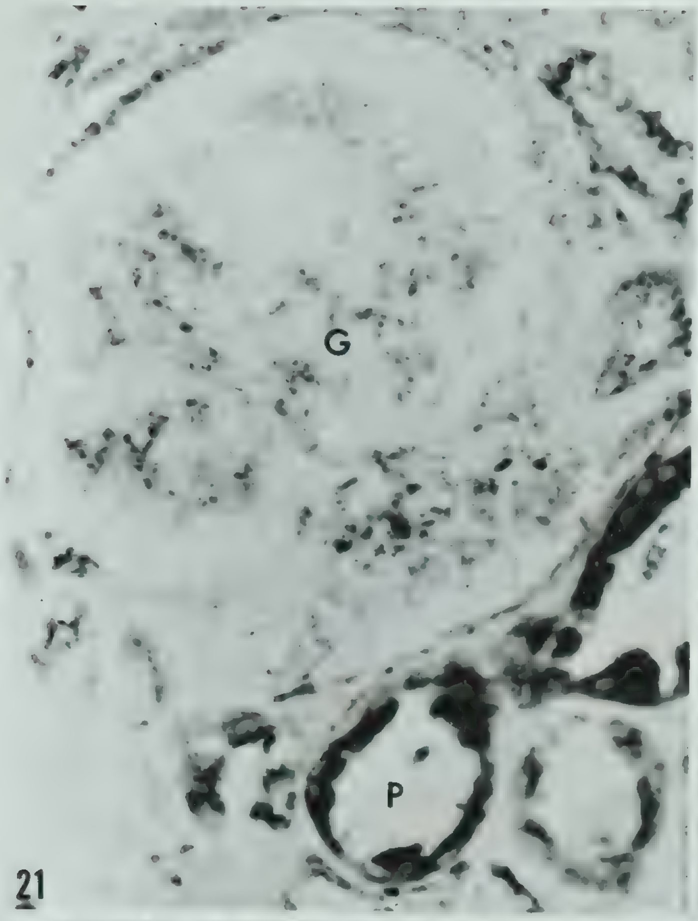


Fig. 21. Man (adult) kidney, a-Naphthyl acetate esterase, note the particulate enzyme activity in the cells of the glomerulus (G) and the high activity in the proximal convoluted tubules (P). 400 X.

Fig. 22. Man (adult) kidney, B-glycerophosphate phosphatase. 250 X.

Fig. 23. Man (adult) kidney, glucosaminidase. 250 X.

Fig. 24. Man (adult) kidney, B-glucuronidase. 250 X.



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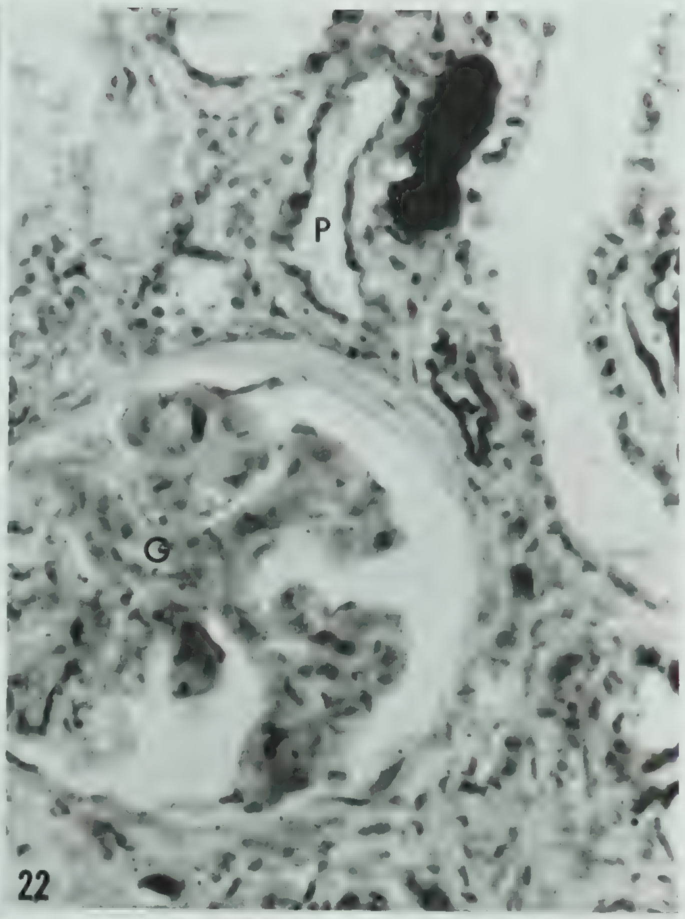

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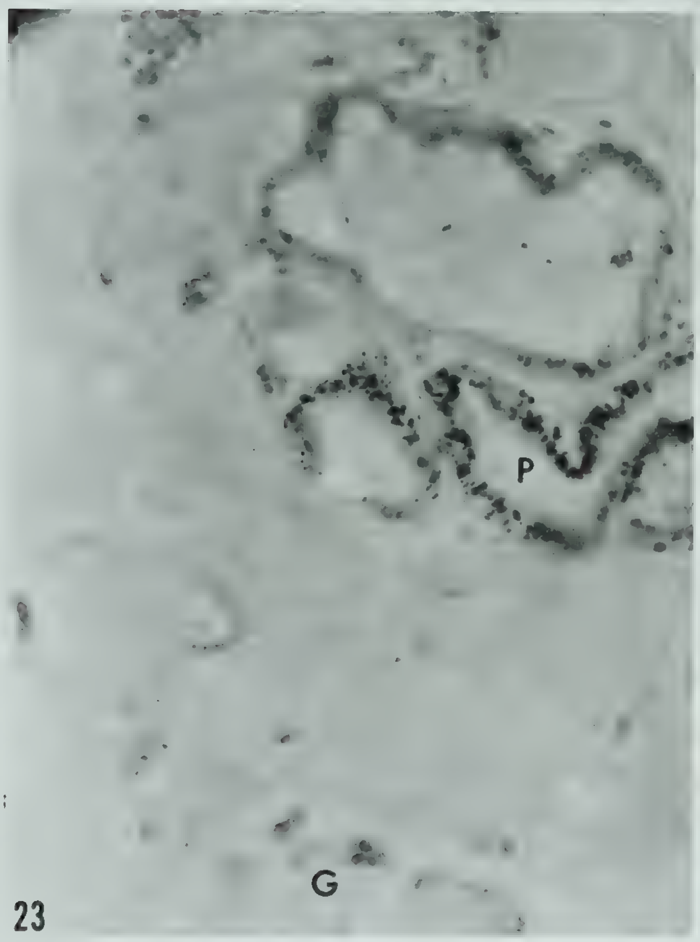

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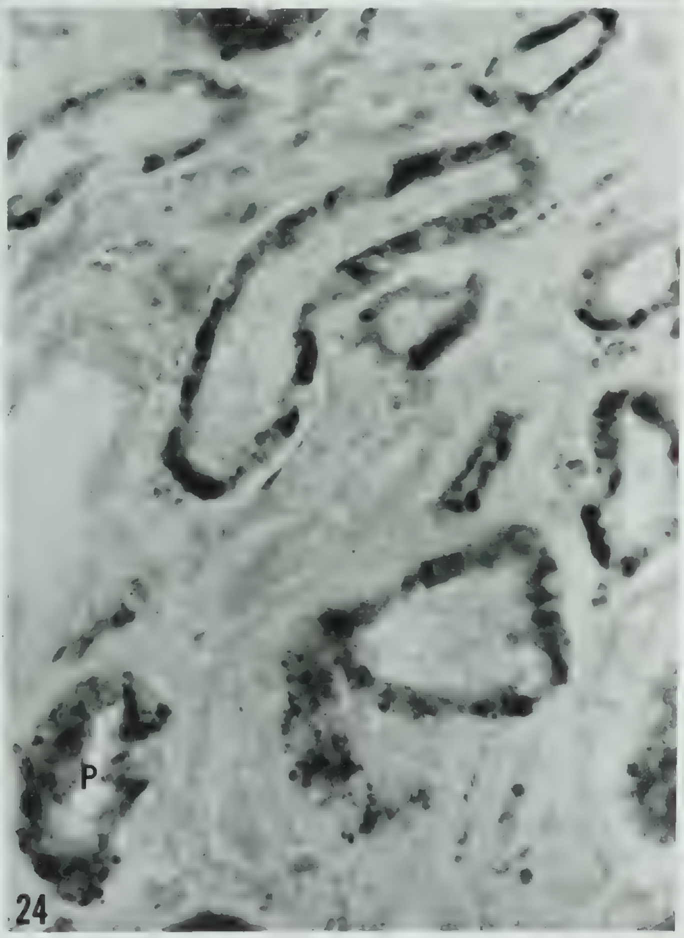

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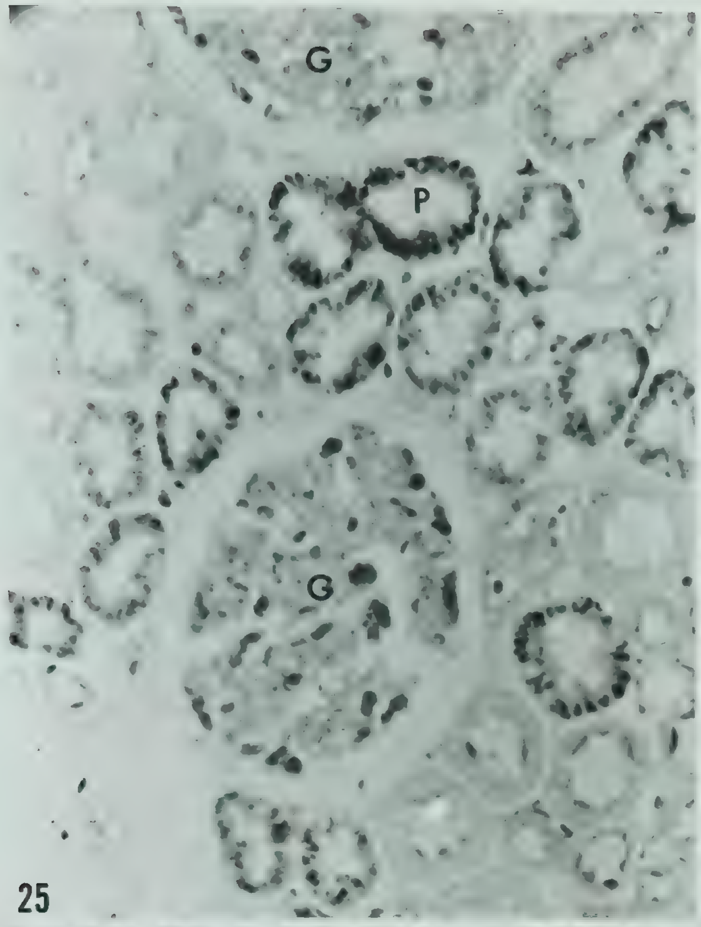
Fig. 25. Dog kidney, glucosaminidase. 250 X.

Fig. 26. Dog kidney, a-Naphthyl acetate esterase. 250 X.

Fig. 27. Man (infant) liver, B-glycerophosphate phosphatase, note the high enzyme activity in Kupffer cells (K), and in the pericanalicular region of parenchymal cells. 250 X.

Fig. 28. Man (adult) liver, B-glycerophosphate phosphatase. 250 X.



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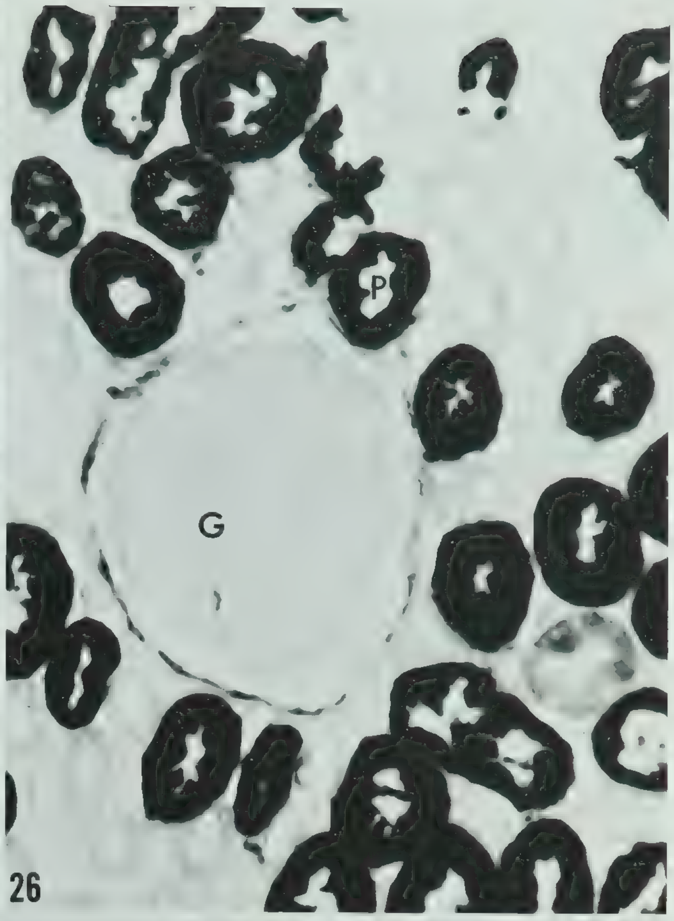

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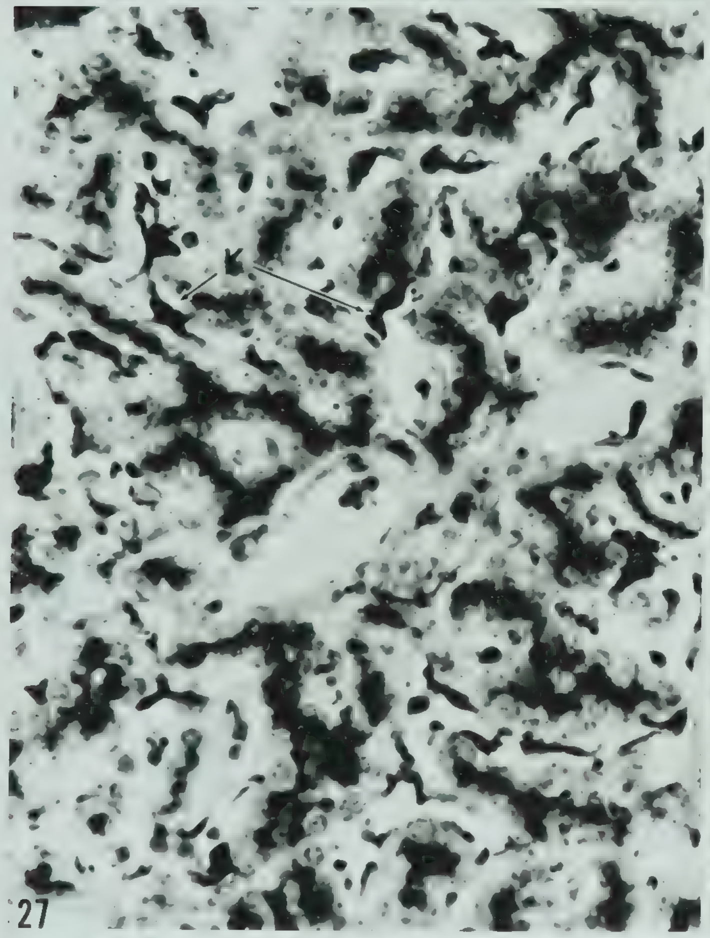

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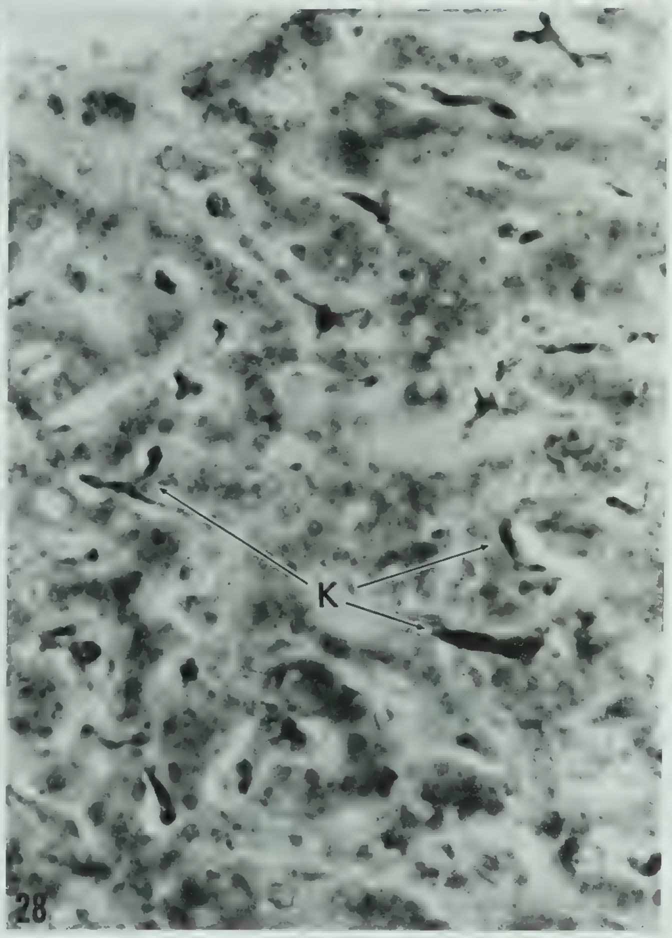

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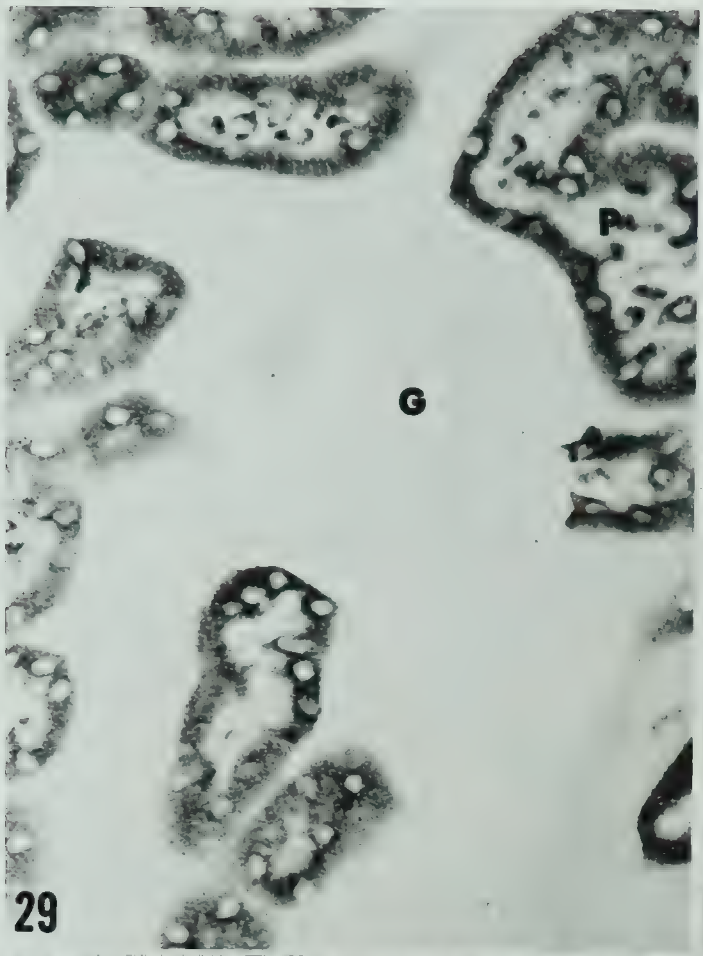
Fig. 29. Rat kidney, Naphthol AS acetate esterase, note the high enzyme activity in the proximal convoluted tubules (P). 250 X.

Fig. 30. Hamster kidney, Naphthol AS acetate esterase. 250 X.

Fig. 31. Hamster liver, B-glycerophosphate phosphatase, note numerous lysosomes (Ly) in the pericanalicular region of parenchymal cells. 250 X.

Fig. 32. Guinea pig liver, B-glycerophosphate phosphatase. 250 X.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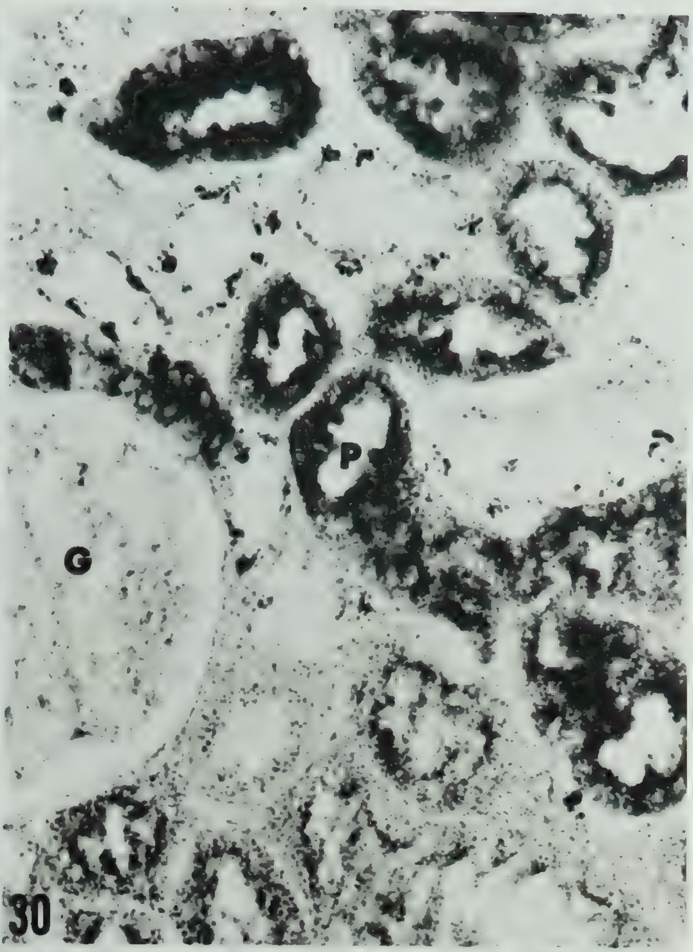
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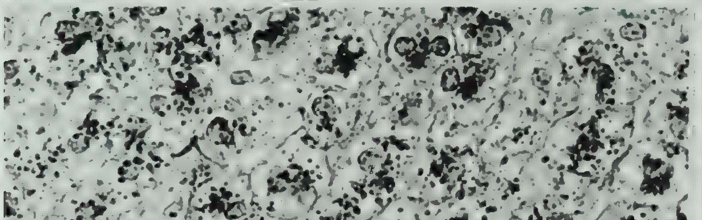
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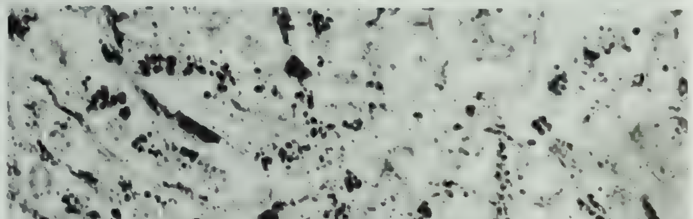
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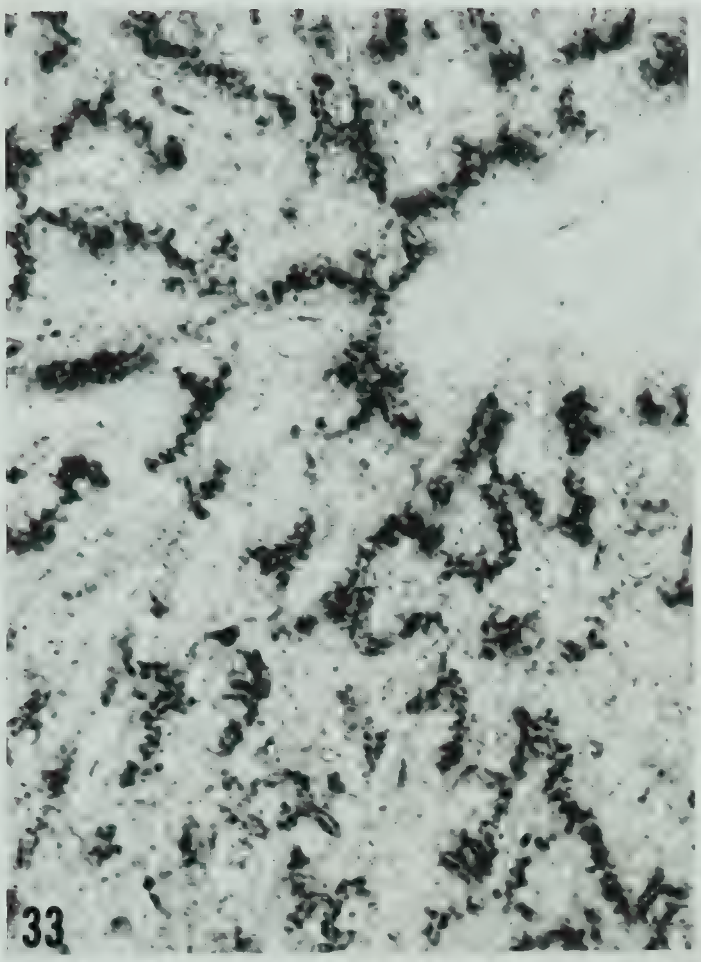
Fig. 33. Horse liver, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 34. Rat liver, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 35. Hamster liver, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 36. Cat liver, Naphthol AS-TR phosphate phosphatase. 250 X.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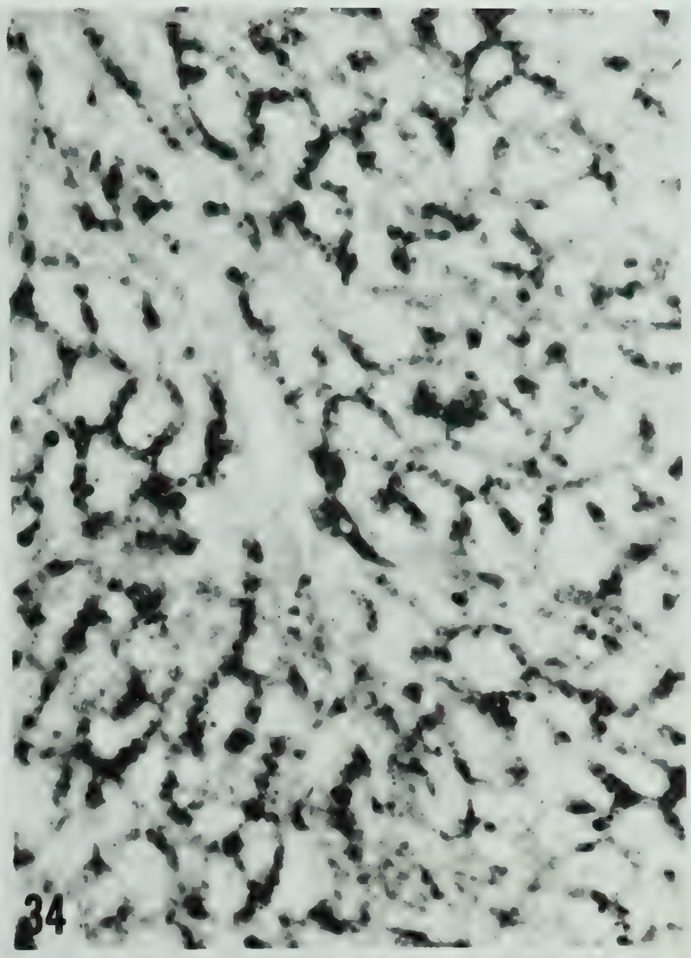
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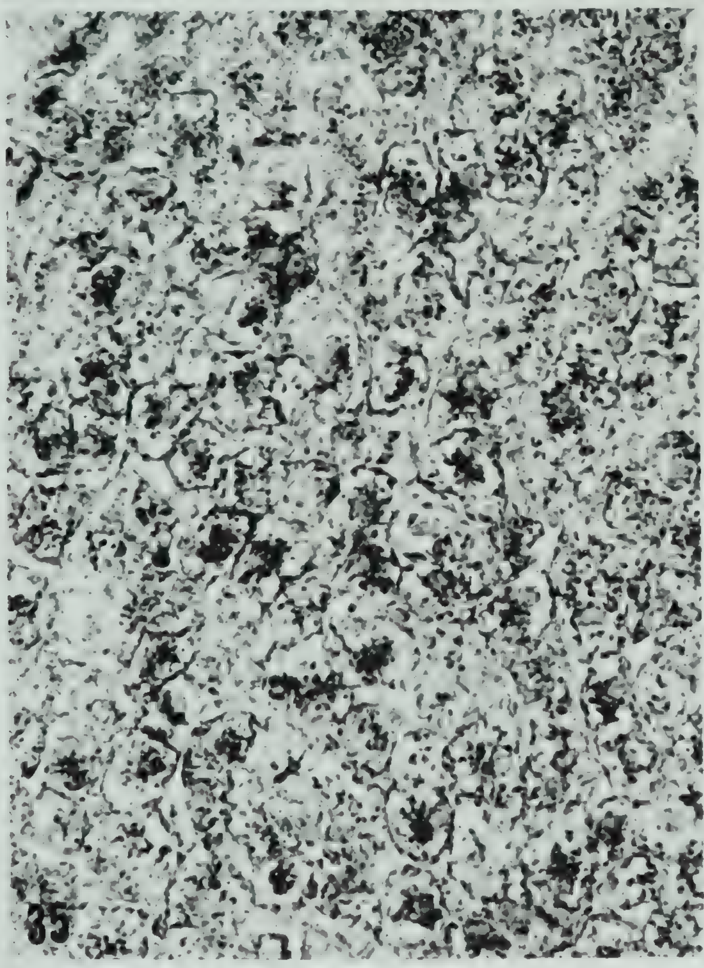
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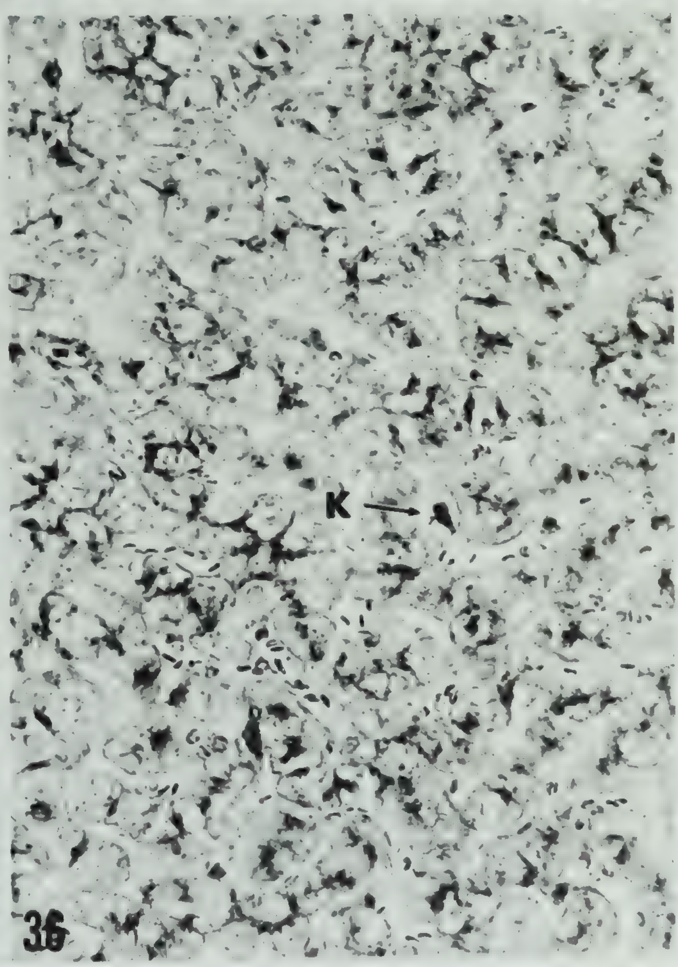
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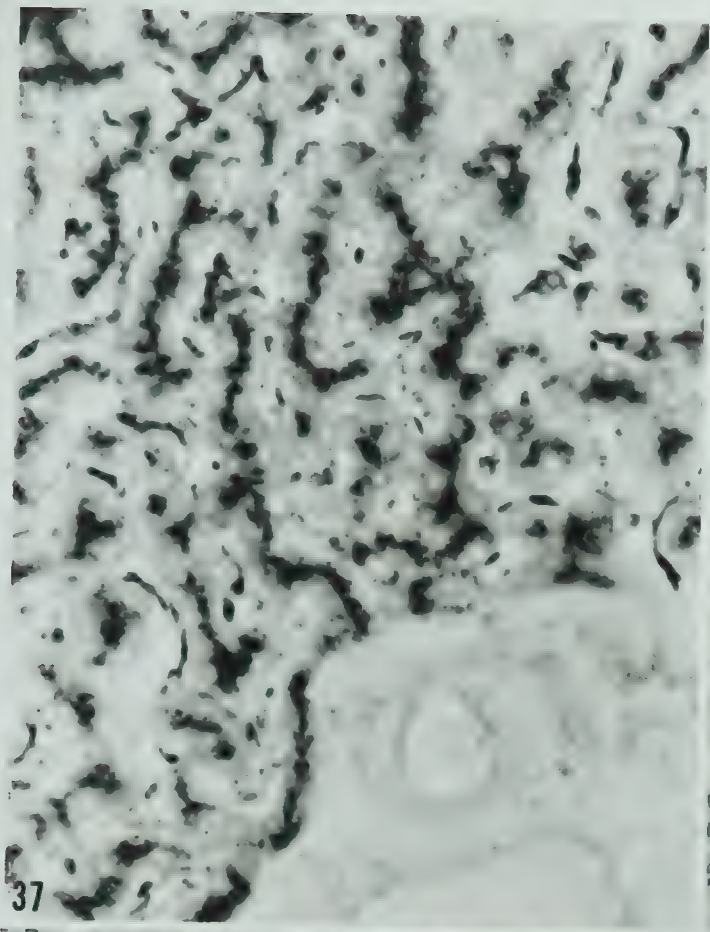
Fig. 37. Man (infant) liver, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 38. Man (infant) liver, B-glucuronidase. 250 X.

Fig. 39. Dog liver, glucosaminidase, note the enzyme localization principally in Kupffer cells (K). 250 X.

Fig. 40. Man (infant) liver, a-Naphthyl acetate esterase. 250 X.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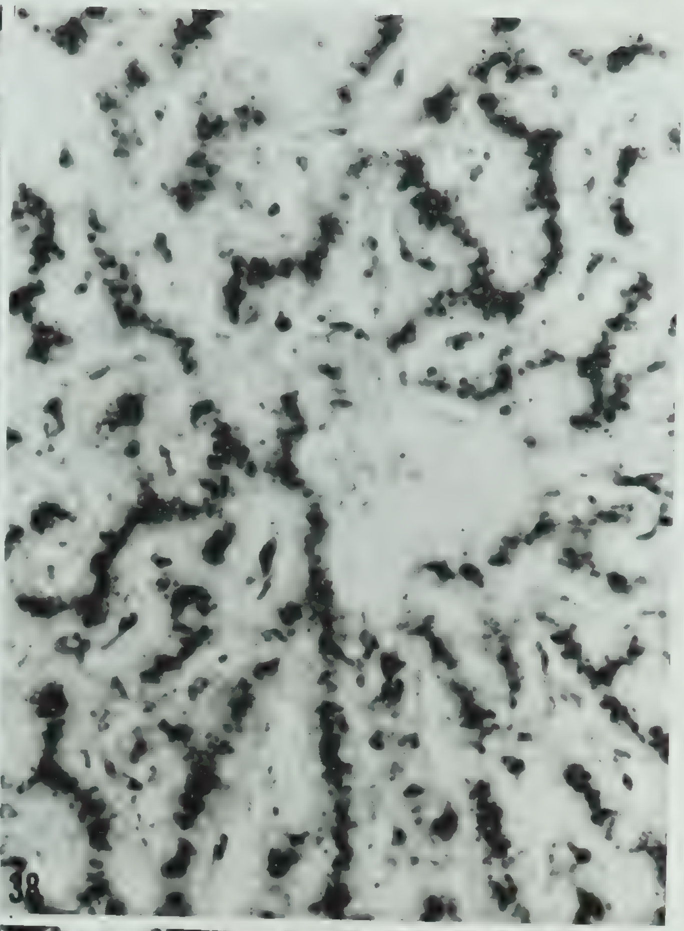
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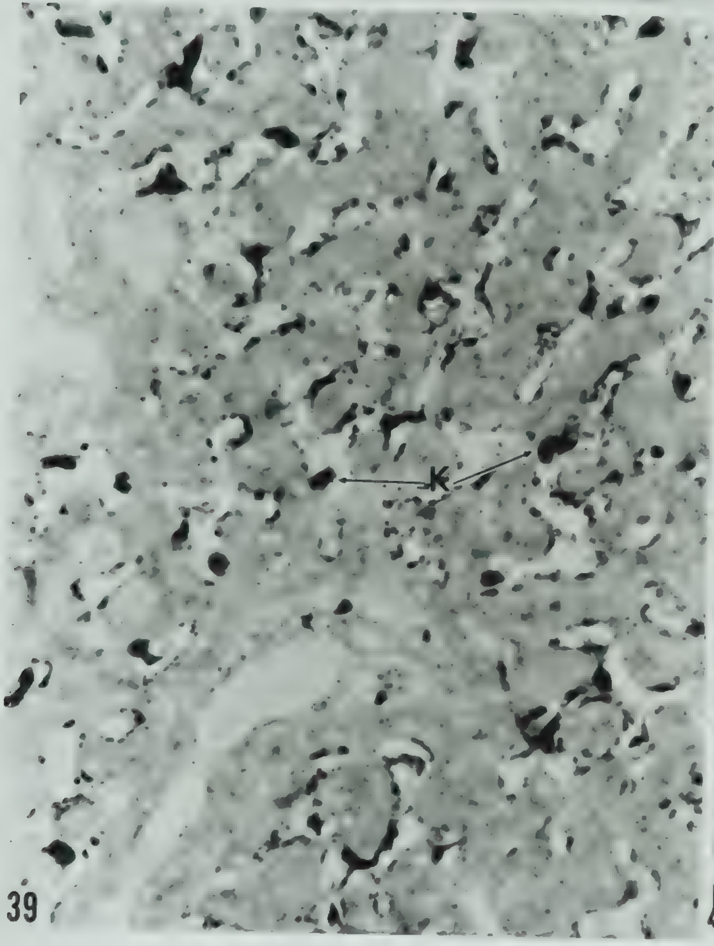

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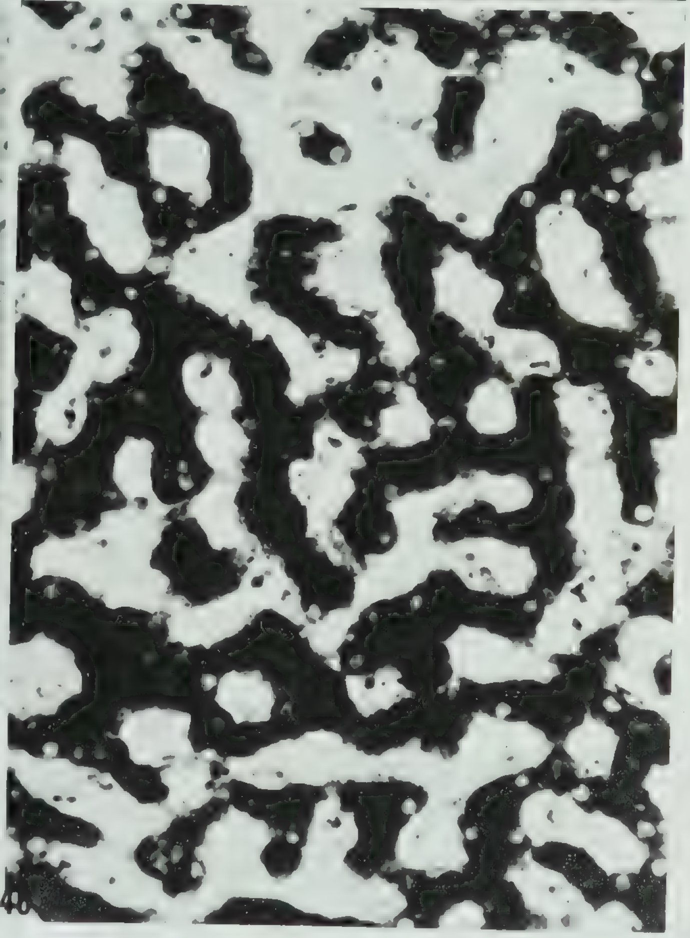
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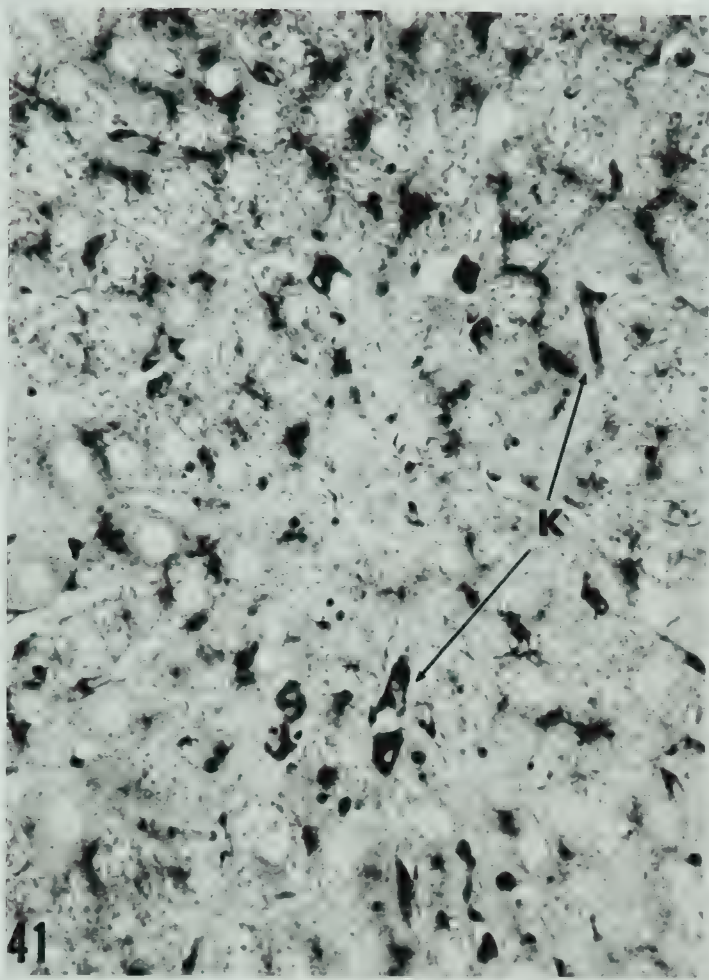
Fig. 41. Rat liver, B-glucuronidase. 250 X.

Fig. 42. Guinea pig liver, B-glucuronidase. 250 X.

Fig. 43. Horse liver, B-glucuronidase. 250 X.

Fig. 44. Sheep liver, B-glucuronidase. 250 X.



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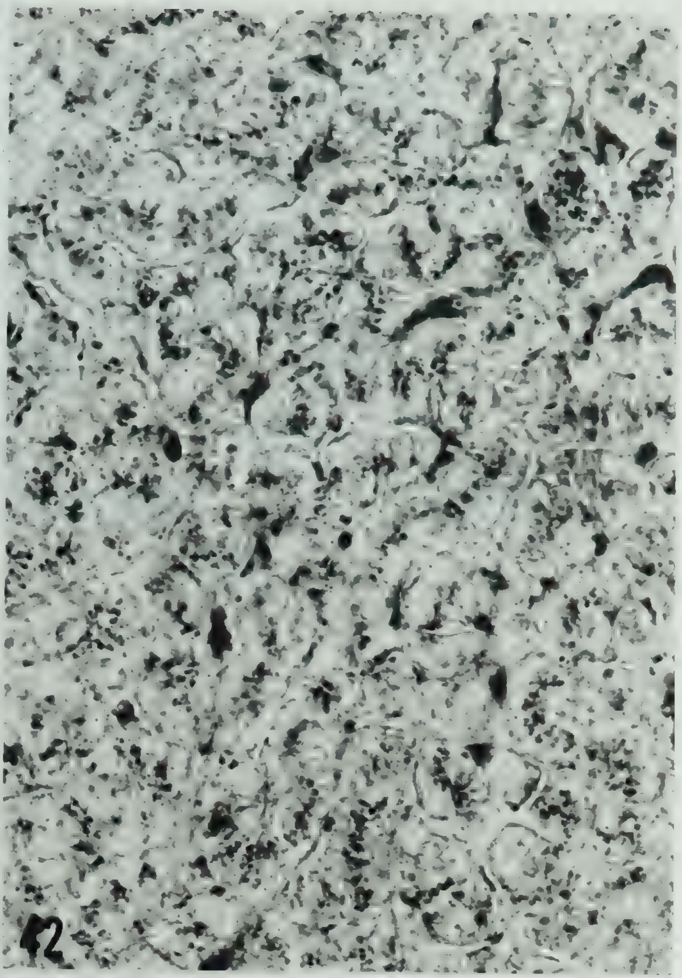

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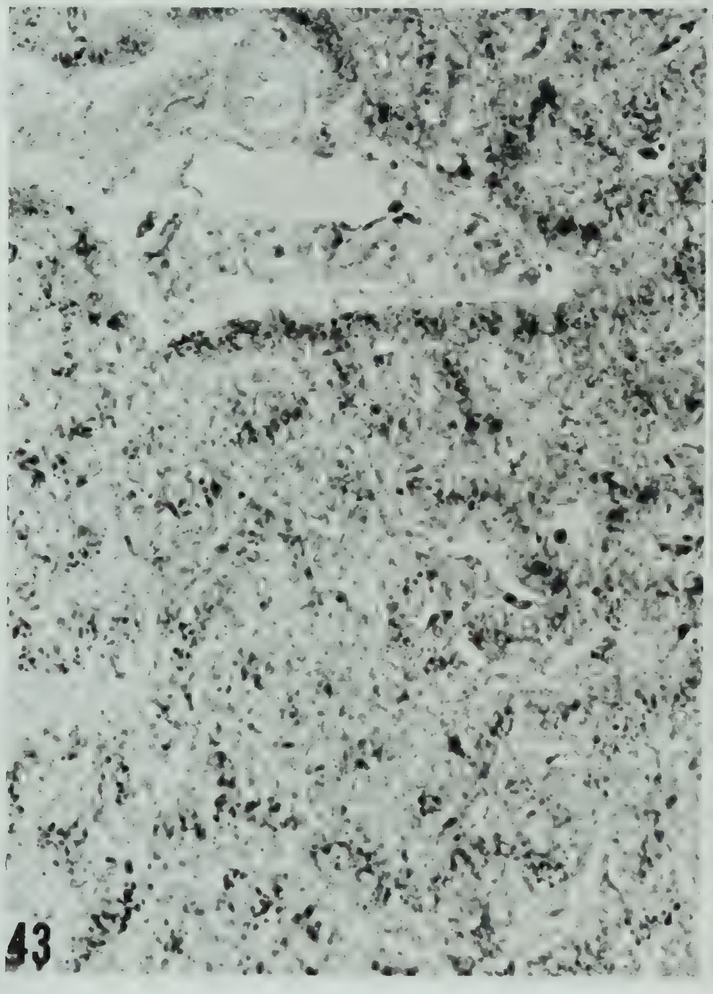

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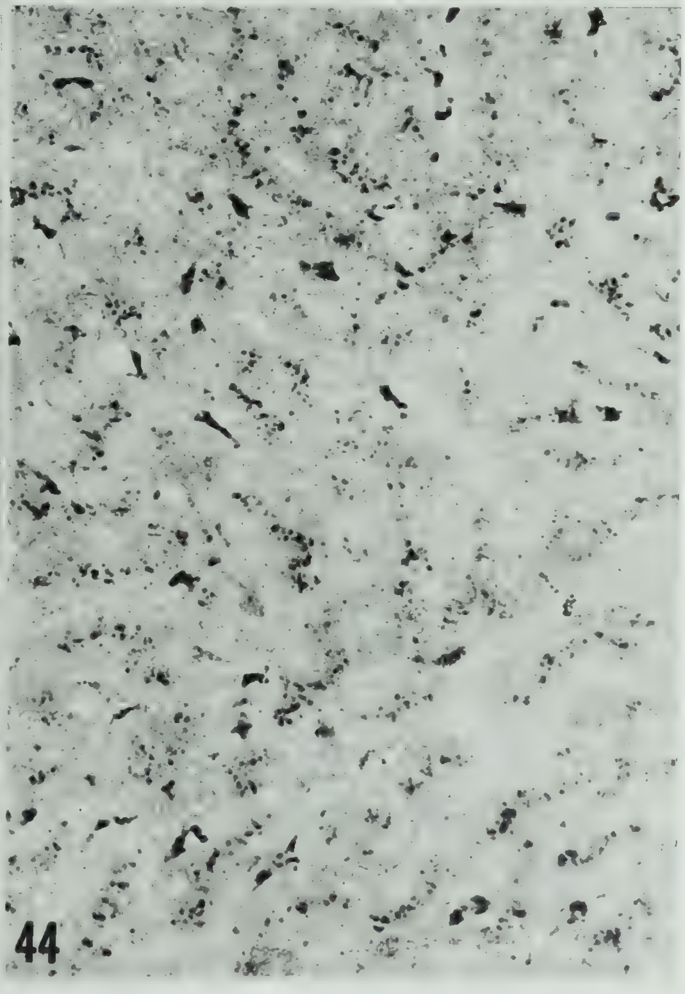

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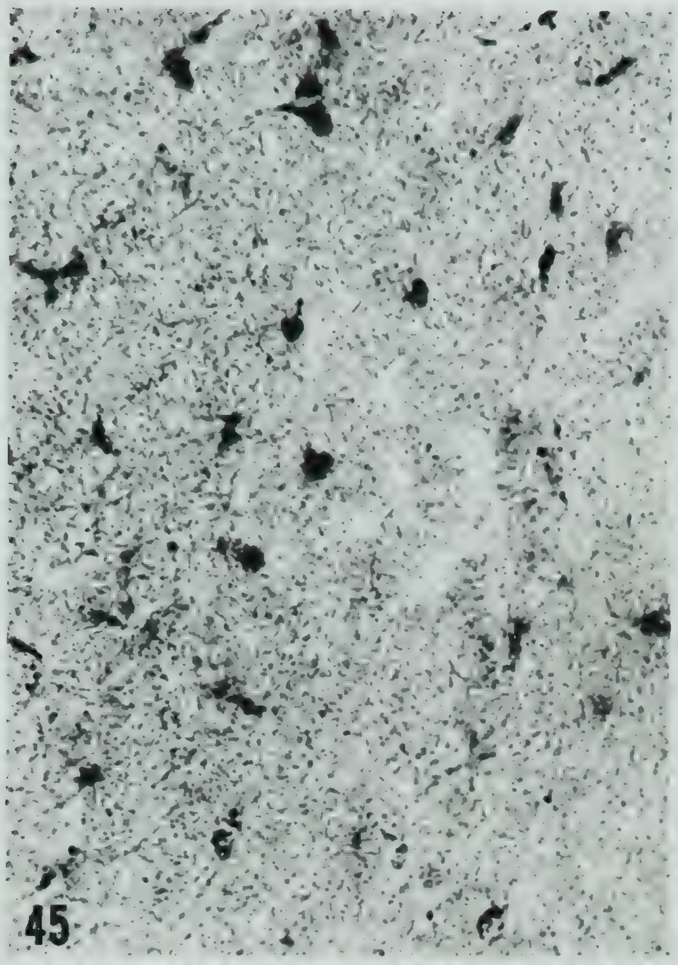
Fig. 45. Hamster liver, B-glucuronidase, note intense enzyme activity in Kupffer cells. 250 X.

Fig. 46. Horse liver, Naphthol AS acetate esterase. 250 X.

Fig. 47. Cat liver, B-glucuronidase, note high enzyme activity in large lysosomes in Kupffer cells (K). 250 X.

Fig. 48. Cat liver, Naphthol AS acetate esterase. 250 X.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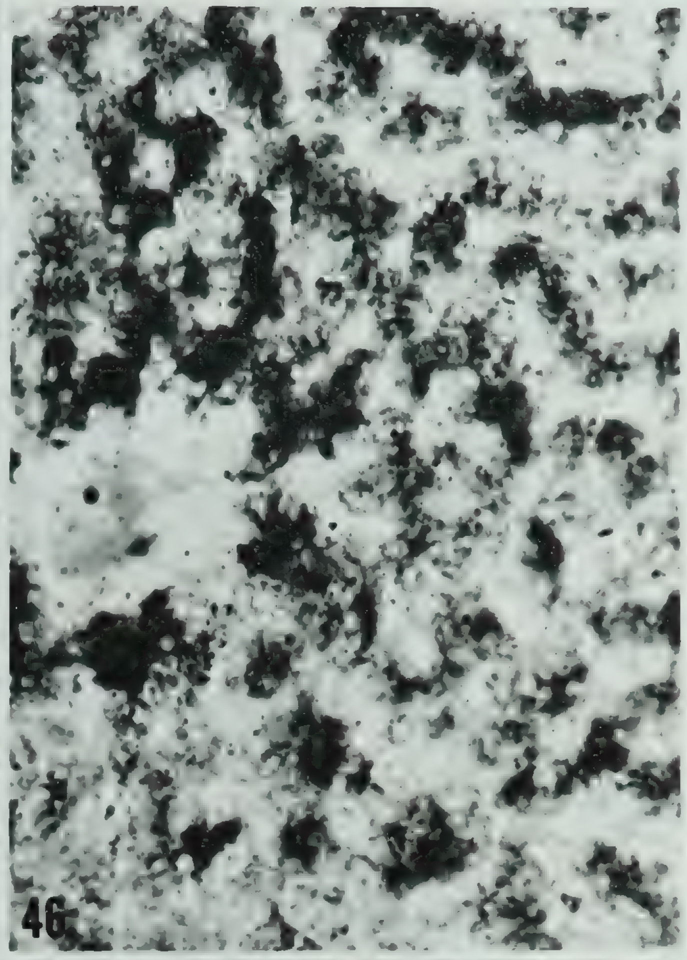
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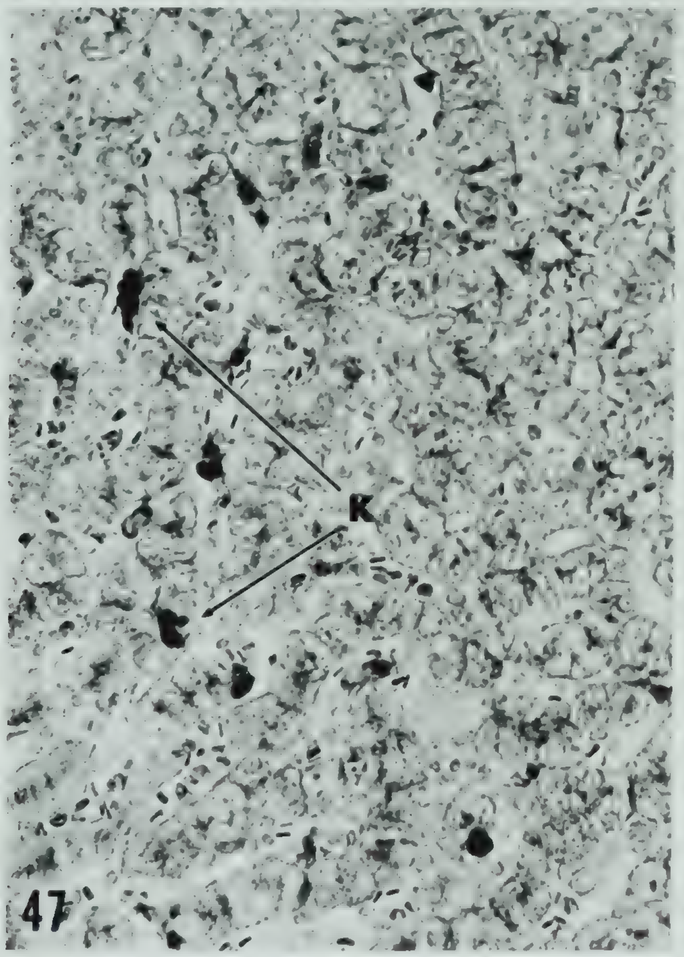
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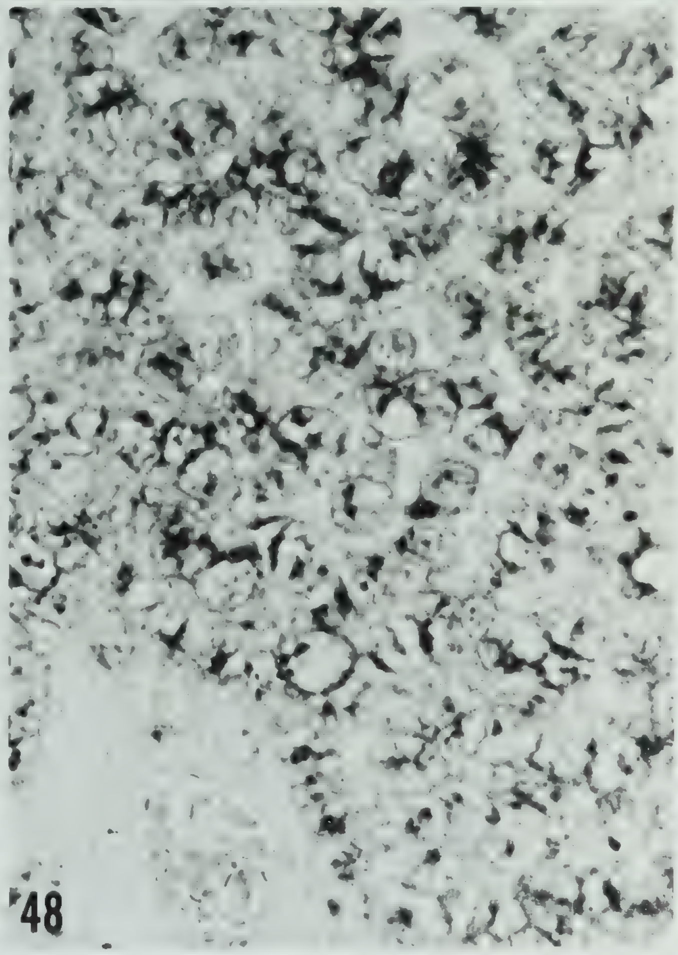
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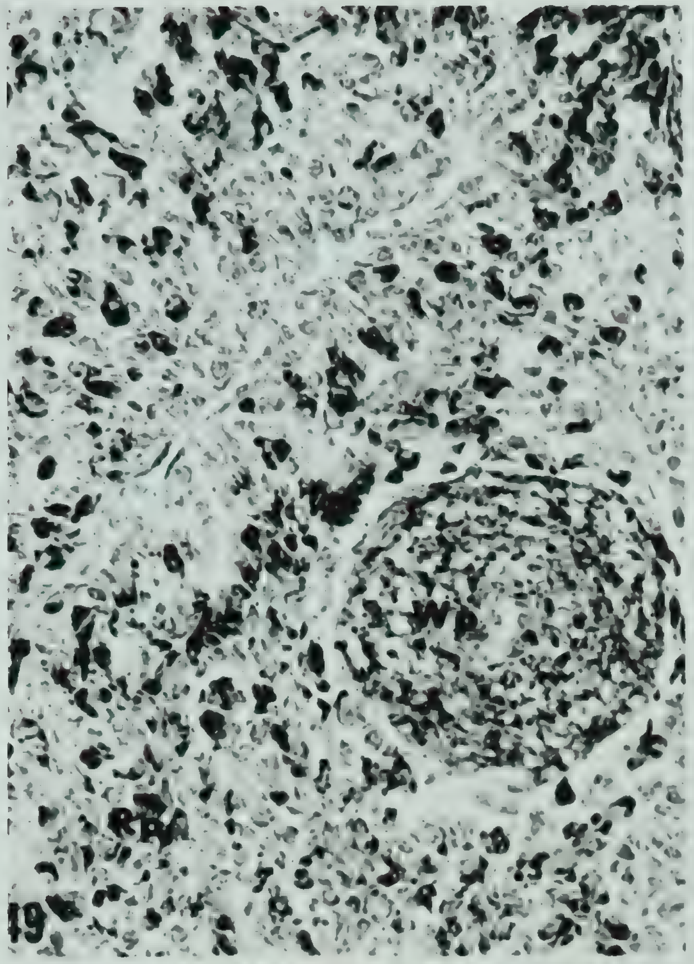
Fig. 49. Pig spleen, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 50. Rat spleen, B-glycerophosphate phosphatase. 250 X.

Fig. 51. Rabbit spleen, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 52. Rabbit spleen, B-glucuronidase. 250 X.



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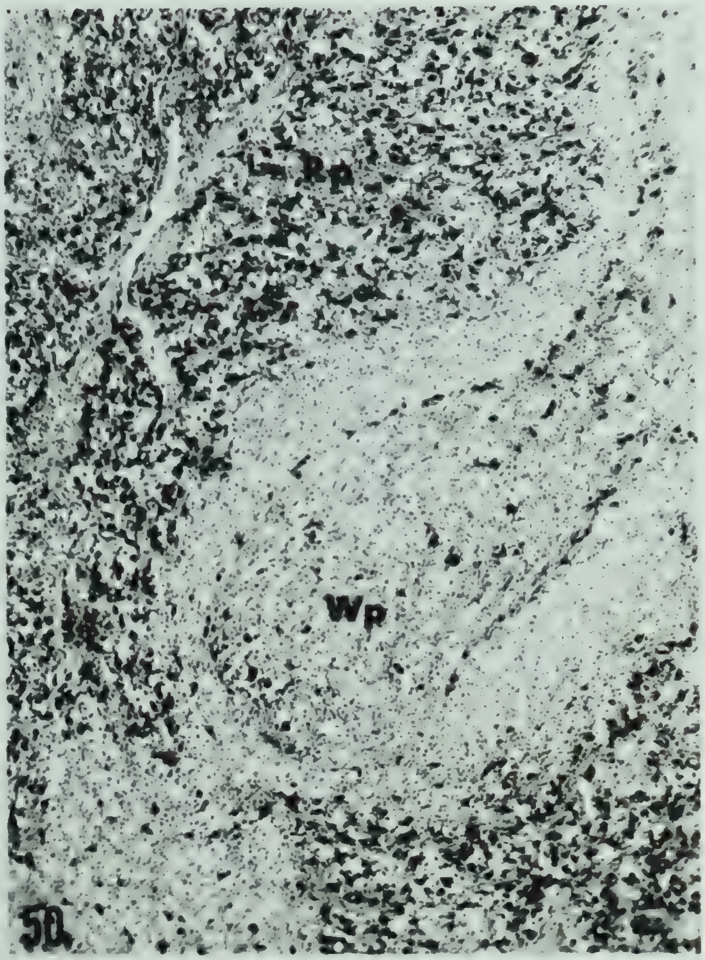

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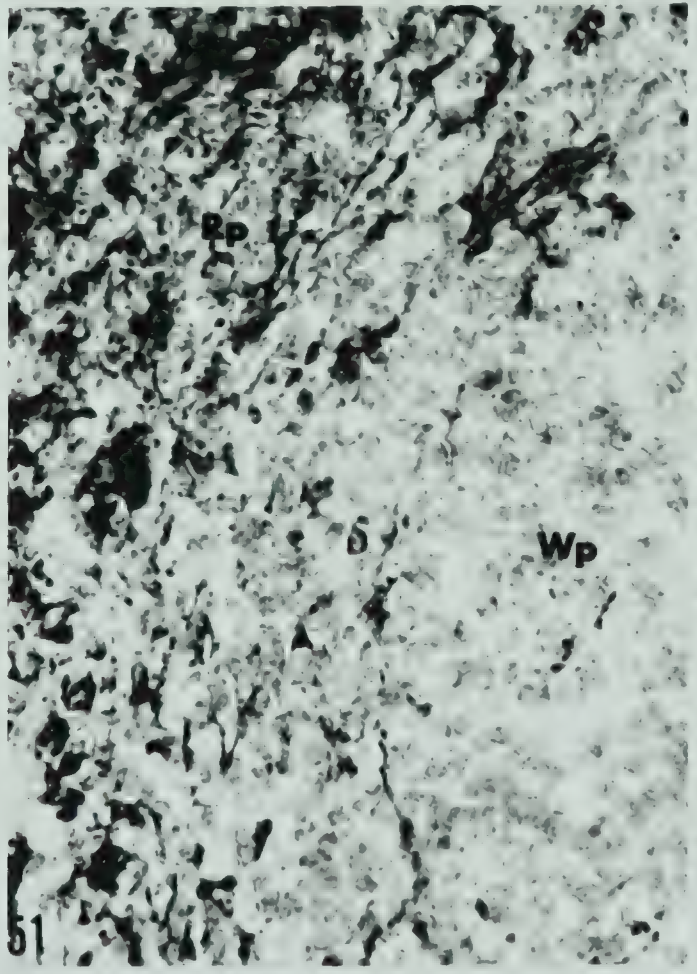

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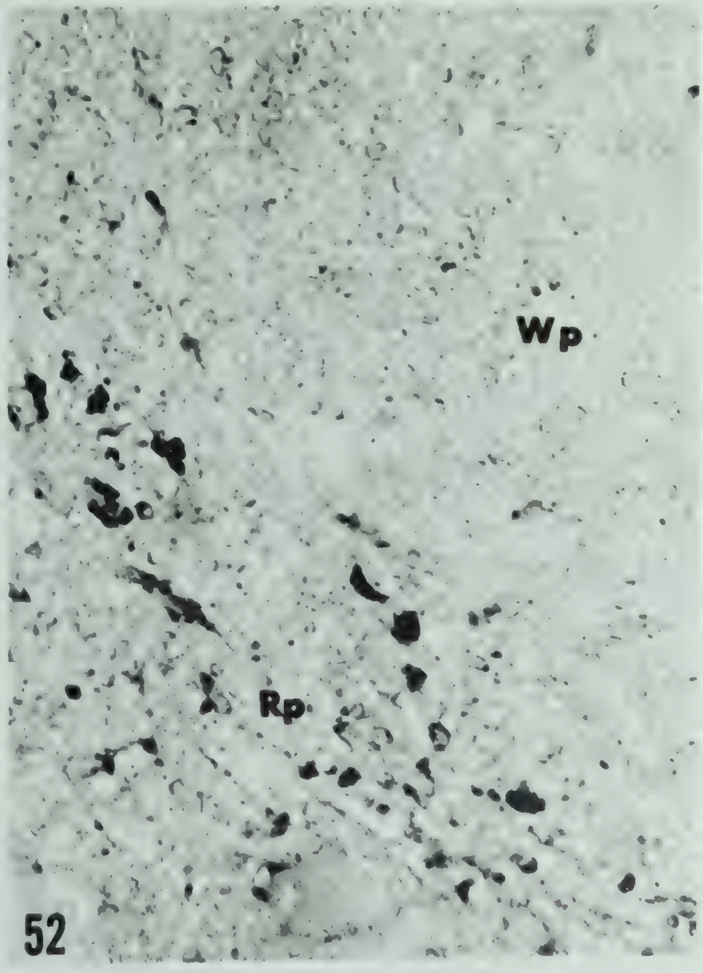


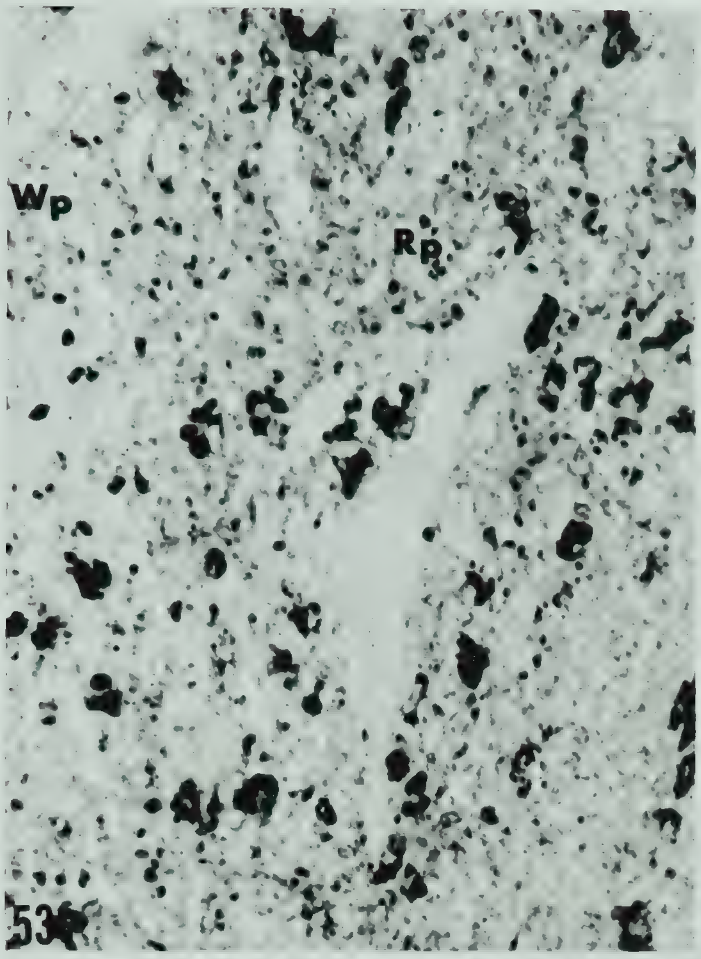
52

Fig. 53. Field mouse spleen, Naphthol AS-TR phosphate phosphatase, note intense enzyme activity in lysosomes of macrophages. 250 X.

Fig. 54. Guinea pig spleen, B-glucuronidase, note intense red pulp macrophage activity for this enzyme. 250 X.

Fig. 55. Field mouse spleen, Naphthol AS acetate esterase. 250 X.

Fig. 56. Horse spleen, Naphthol AS-TR phosphate phosphatase, note high enzyme activity in lysosomes of macrophages of the red pulp (Rp). 250 X.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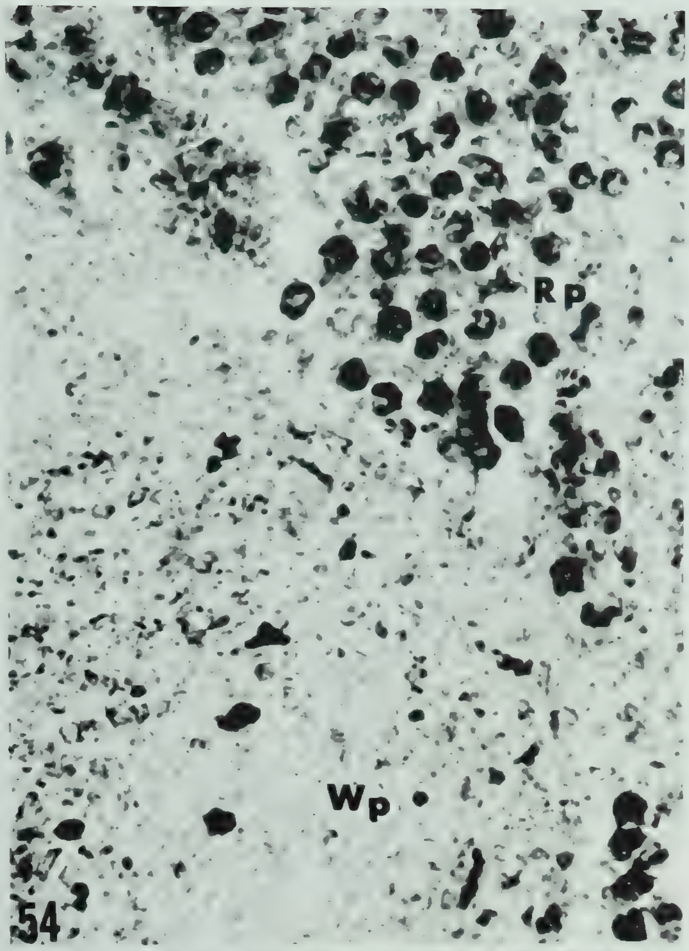
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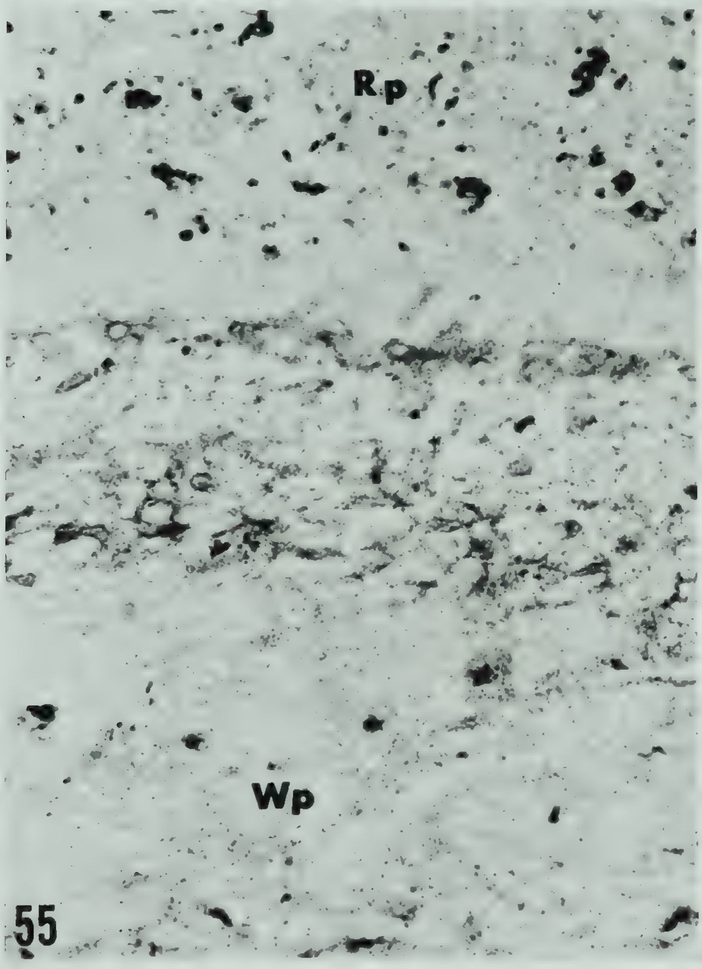
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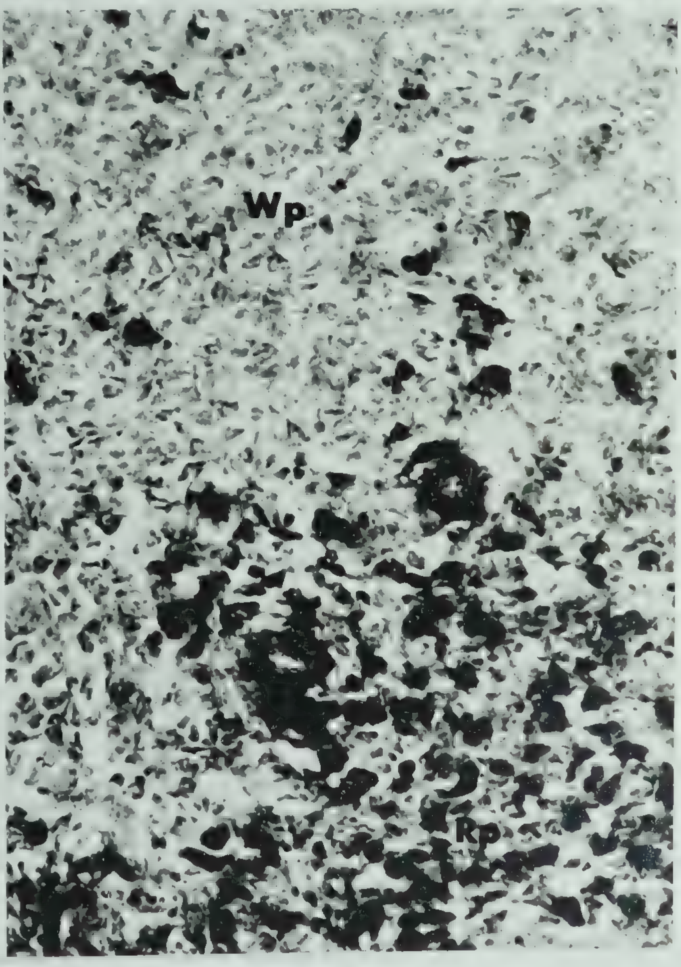
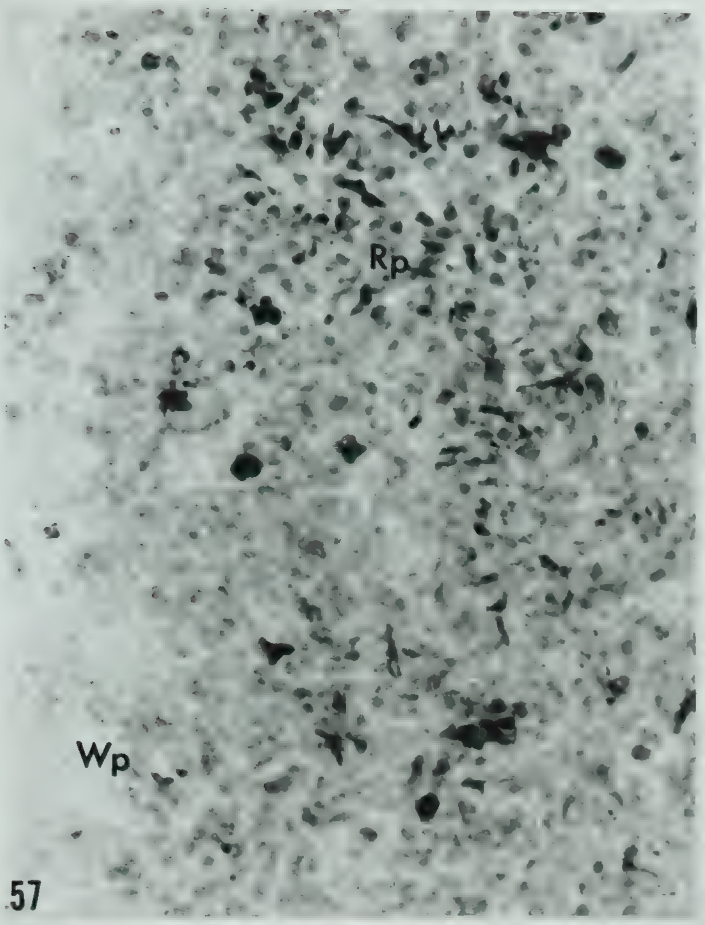


Fig. 57. Man (adult) spleen, B-glycerophosphate phosphatase. 250 X.

Fig. 58. Man (infant) spleen, B-glycerophosphate phosphatase, note high enzyme activity in the red pulp (Rp) but absence of this activity in the white pulp (Wp). 250 X.

Fig. 59. Man (infant) pancreas, B-glycerophosphate phosphatase, note high enzyme activity in acinar cells (A) and duct epithelium (D). 250 X.

Fig. 60. Man (infant) pancreas, Naphthol AS-TR phosphate phosphatase, note intense enzyme activity in lysosomes of acinar cells (A) and Islet of Langerhans (I). 250 X.



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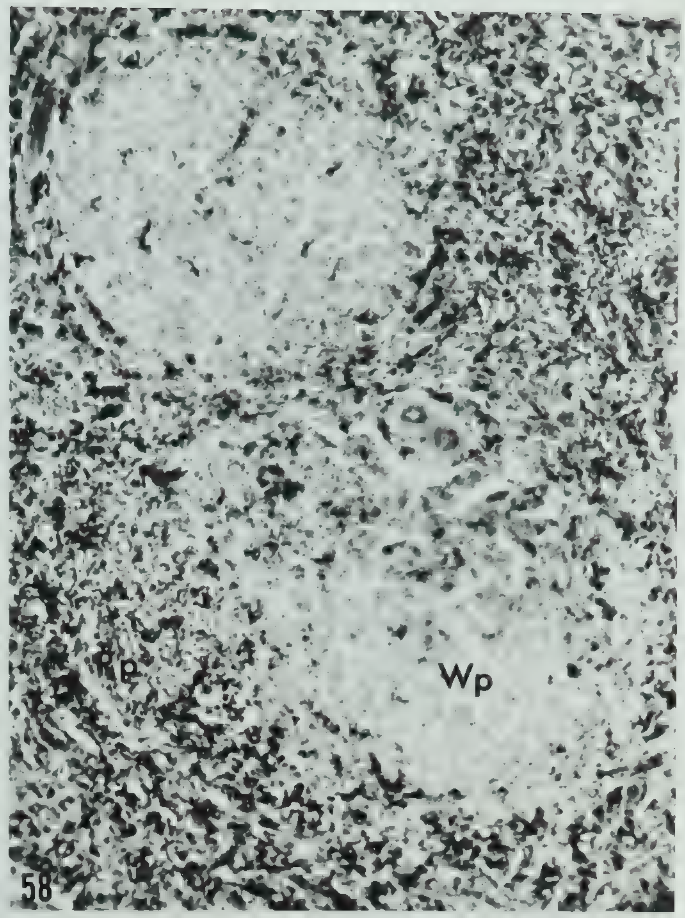

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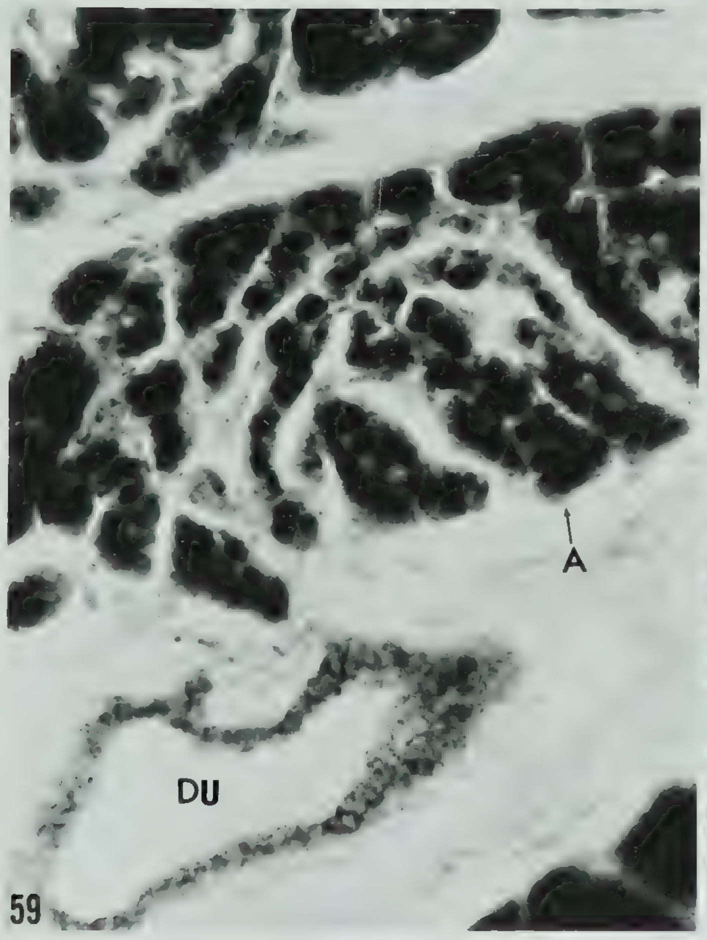

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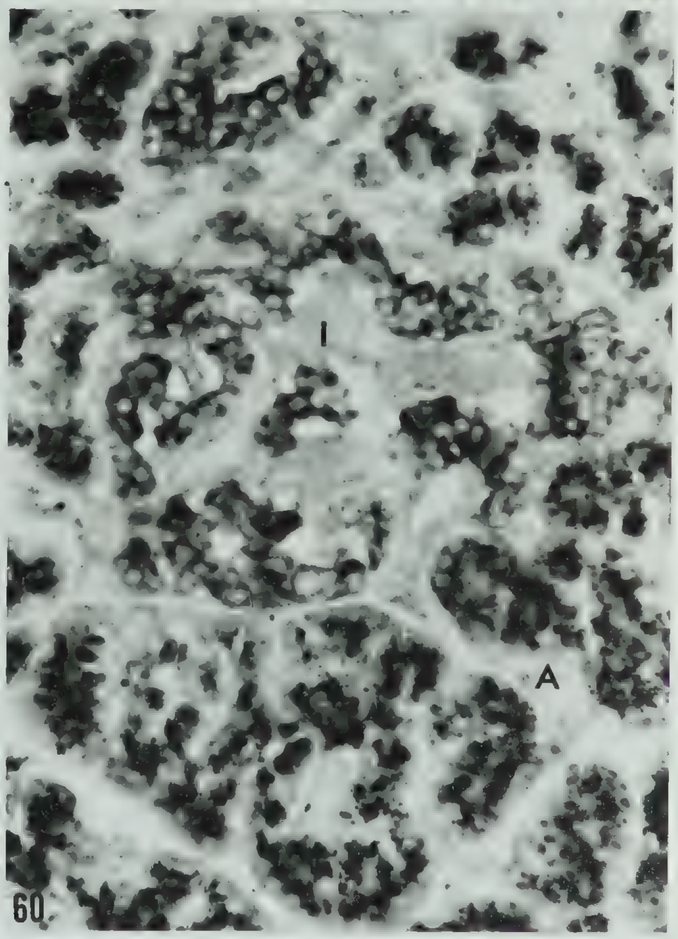

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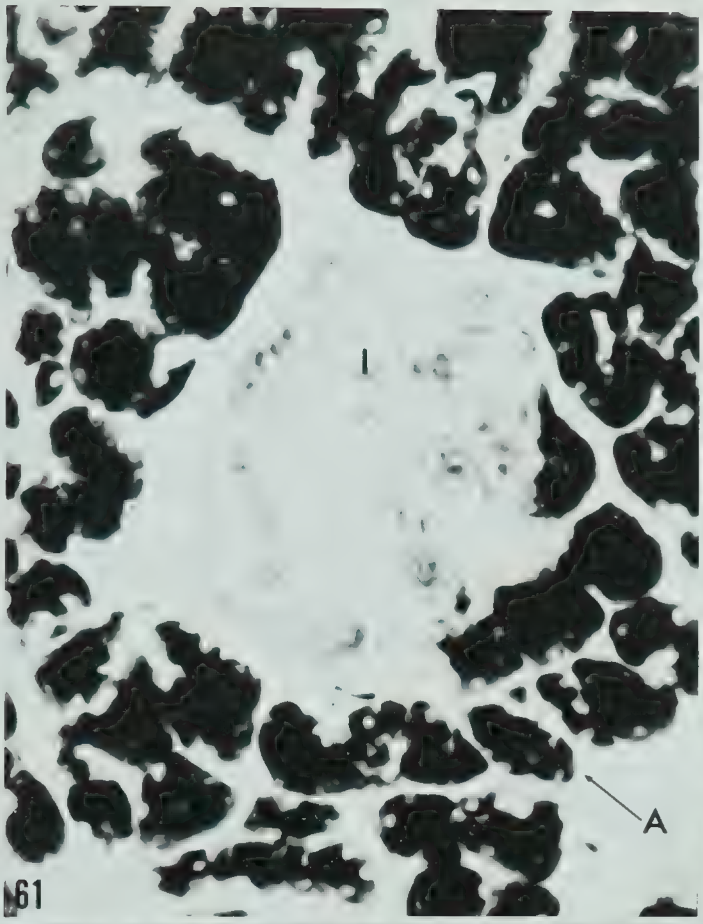
Fig. 61. Man (infant) pancreas, a-Naphthyl acetate esterase, note the slight enzyme activity in the Islet of Langerhans (I). 250 X.

Fig. 62. Dog pancreas, a-Naphthyl acetate esterase. 250 X.

Fig. 63. Dog pancreas, glucosaminidase, note high enzyme activity in duct epithelium (D). 250 X.

Fig. 64. Man (infant) pancreas, Naphthol AS acetate esterase. 250 X.



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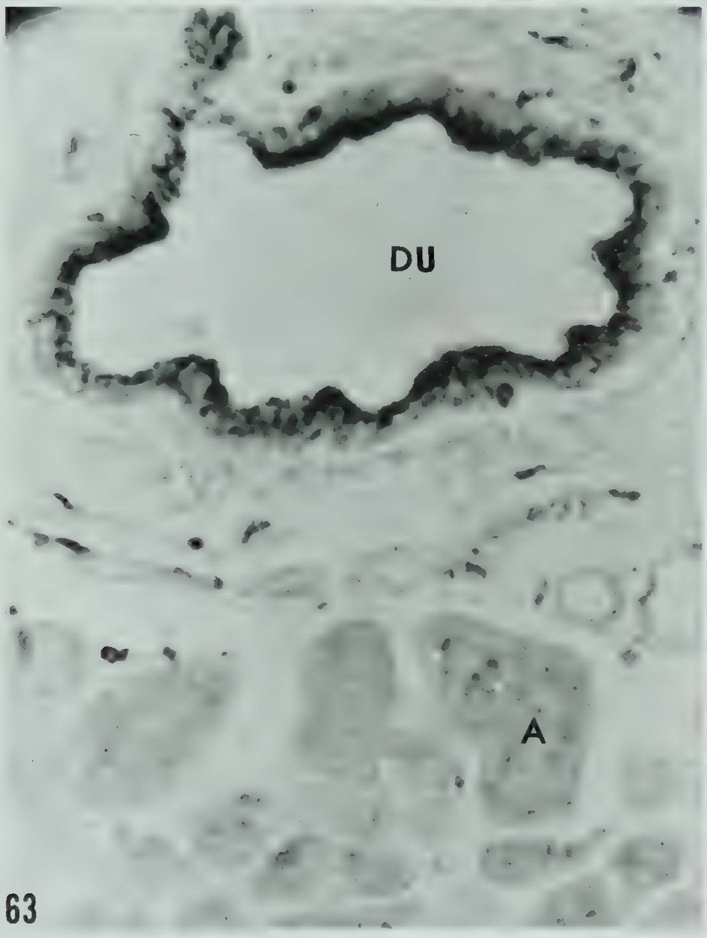

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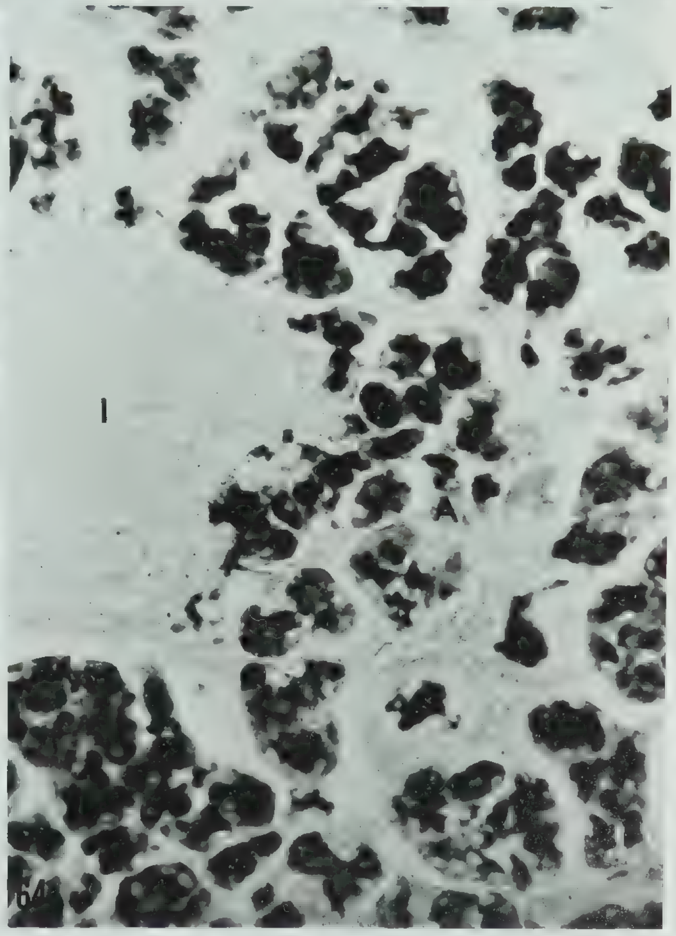

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Fig. 65. Dog pancreas, B-glycerophosphate phosphatase. 250 X.

Fig. 66. Cat pancreas, a-Naphthyl acetate esterase. 250 X.

Fig. 67. Rat pancreas, Naphthol AS acetate esterase, note enzyme activity in cells at periphery of the Islet of Langerhans (I). 250 X.

Fig. 68. Guinea pig duodenum, B-glycerophosphate phosphatase, note intense enzyme activity in lysosomes of the lamina propria (Lp) and mucosal epithelium (Mu). 250 X.

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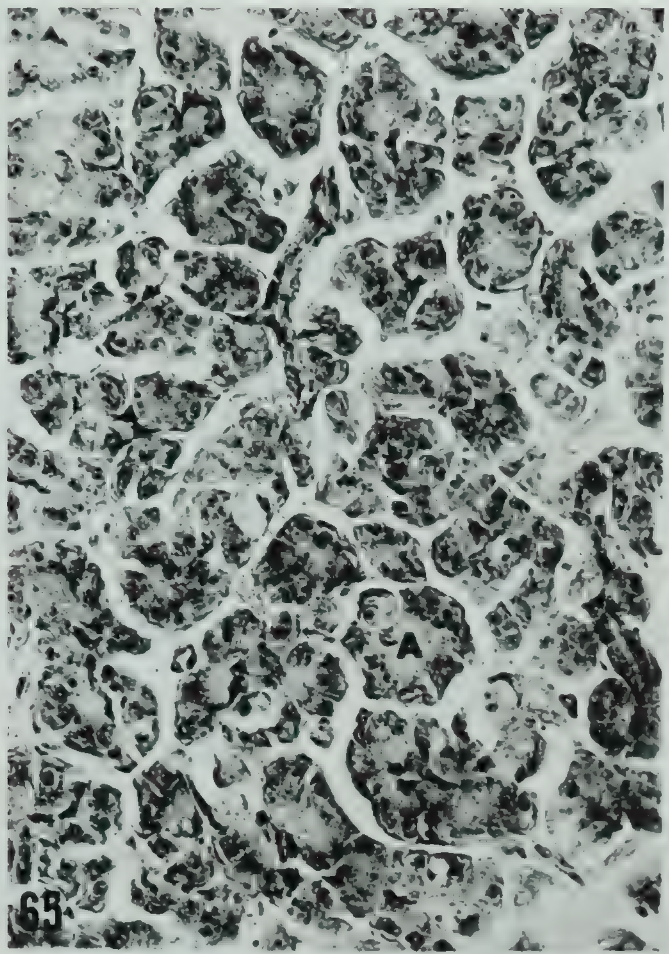

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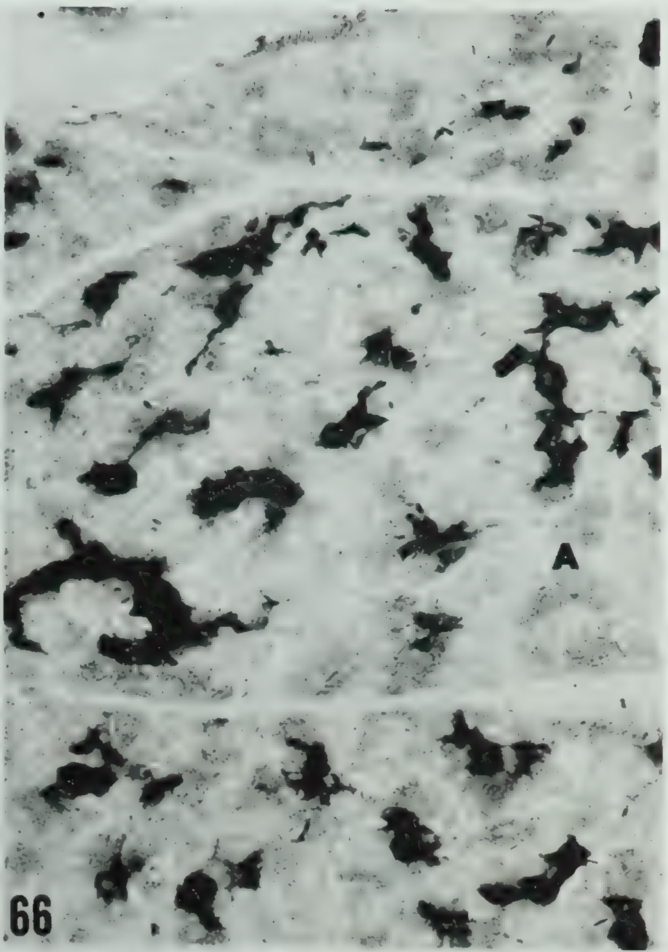

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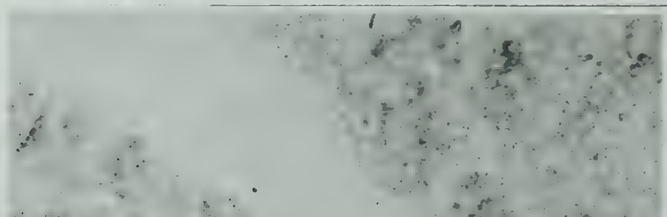

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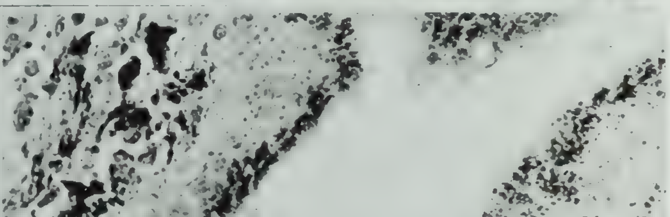
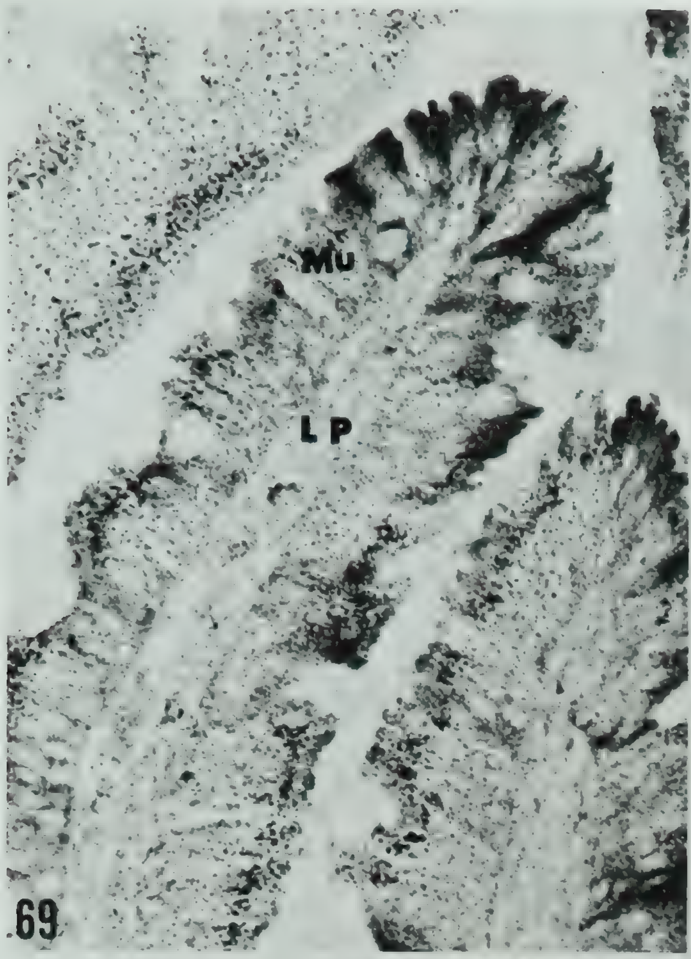


Fig. 69. Pig duodenum, Naphthol AS acetate esterase, note the fine granularity of the reaction product. 250 X.

Fig. 70. Horse duodenum, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 71. Pig duodenum, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 72. Rat duodenum, B-glucuronidase, note intense enzyme activity in the lysosomes of the macrophages in the lamina propria only. 250 X.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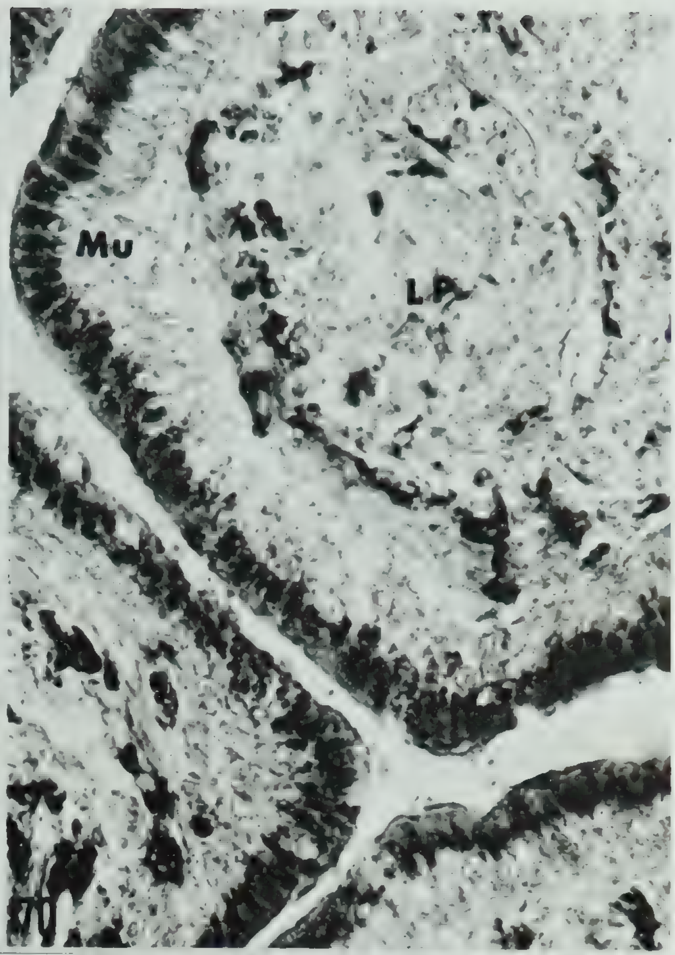
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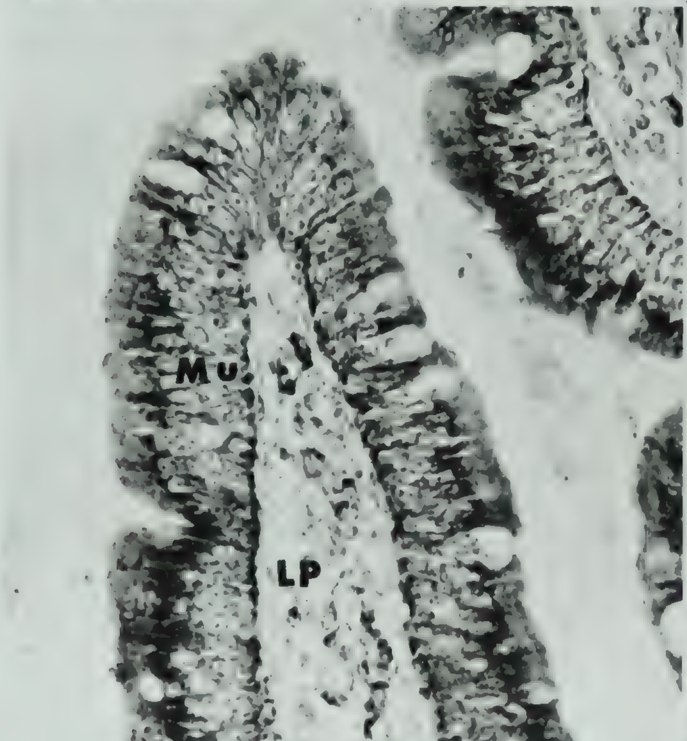
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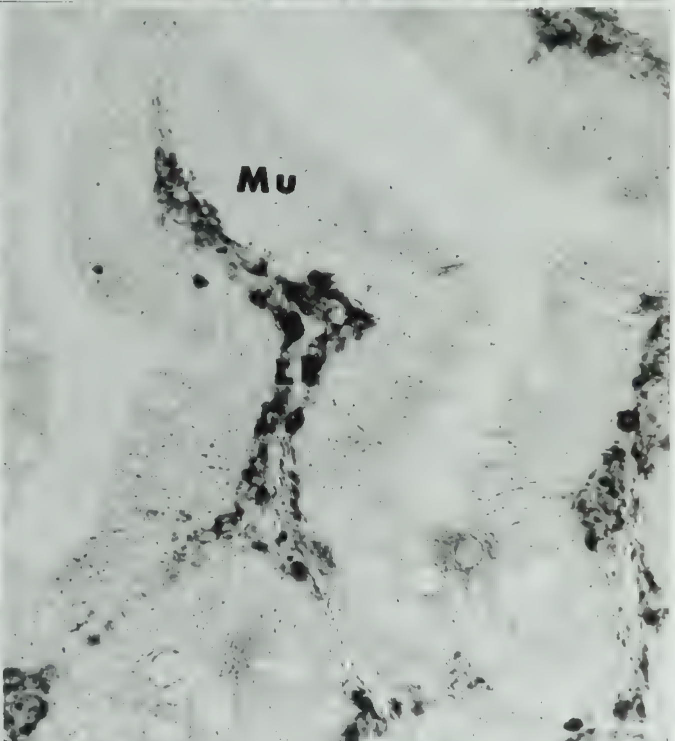
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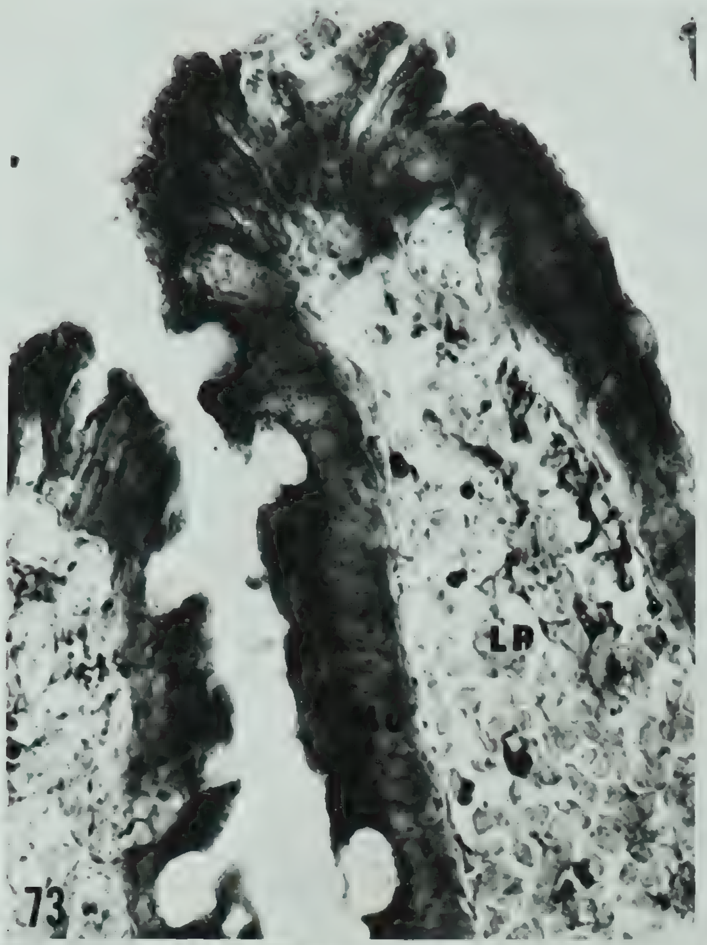
Fig. 73. Hamster duodenum, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 74. Guinea pig duodenum, glucosaminidase. 250 X.

Fig. 75. Hamster duodenum, B-glucuronidase. 250 X.

Fig. 76. Cat duodenum, glucosaminidase. 250 X.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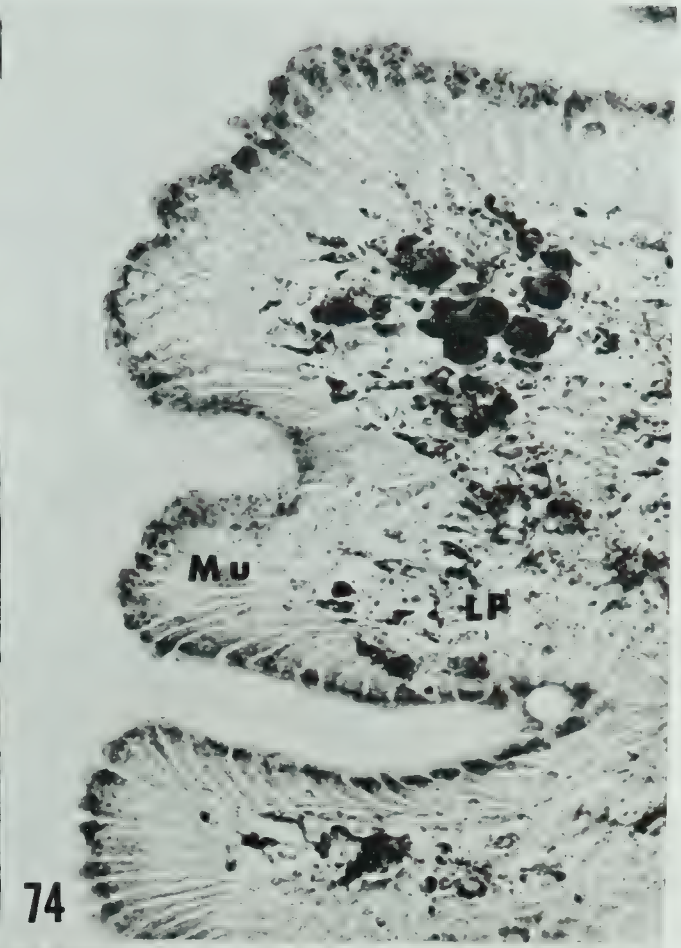
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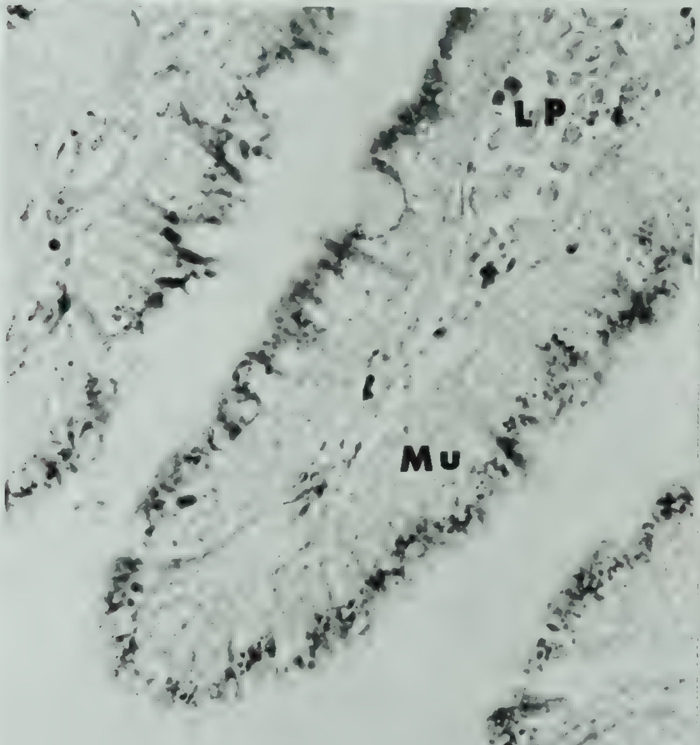
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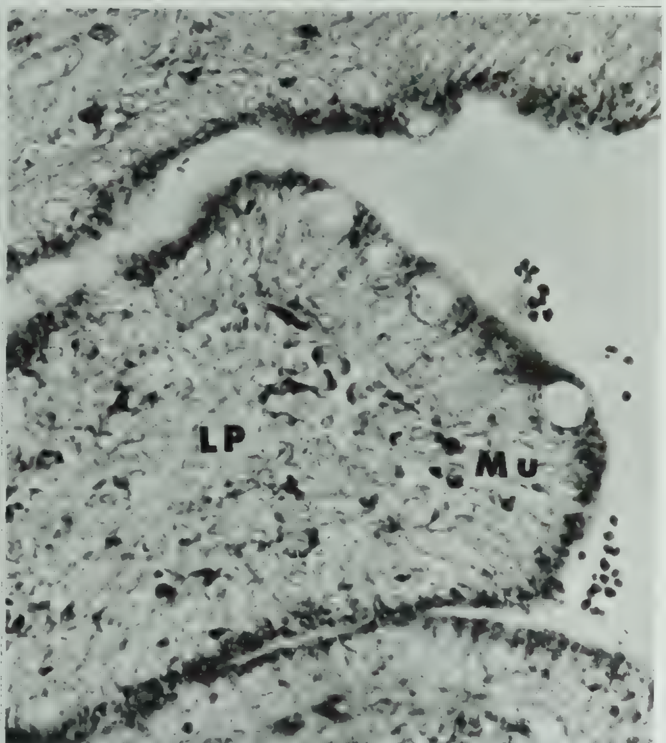
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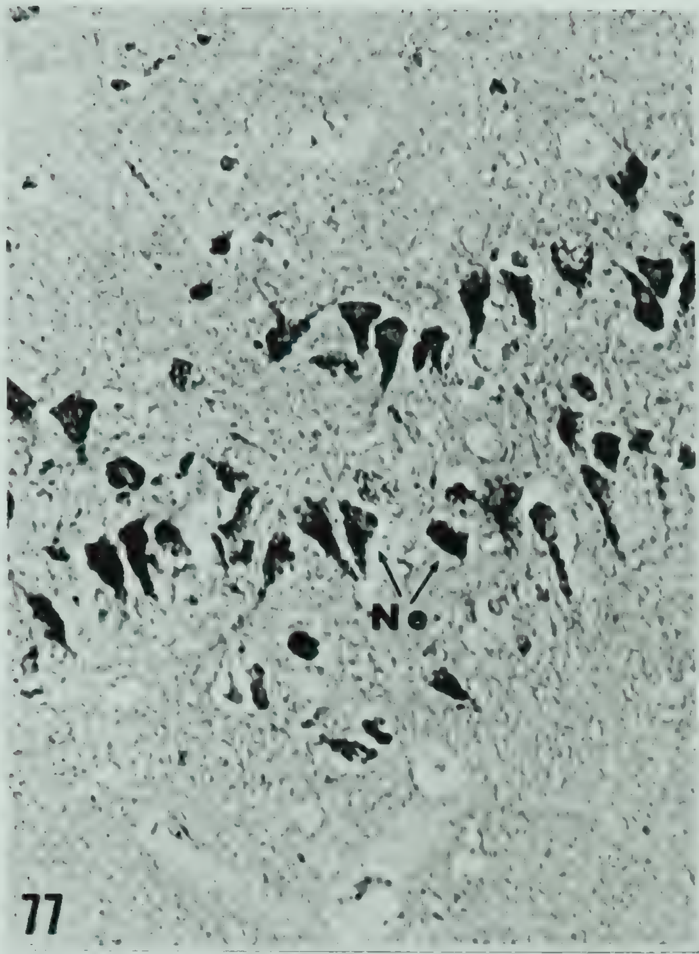
Fig. 77. Hamster brain, B-glycerophosphate phosphatase, note the intense enzyme activity in the lysosomes of the neurons (Ne). 250 X.

Fig. 78. Rabbit brain, B-glycerophosphate phosphatase. 250 X.

Fig. 79. guinea pig brain, glucosaminidase. 250 X.

Fig. 80. Rat brain, Naphthol AS acetate esterase, note the enzyme activity in the neurons (Ne) and perithelial microglia (Pm). 250 X.



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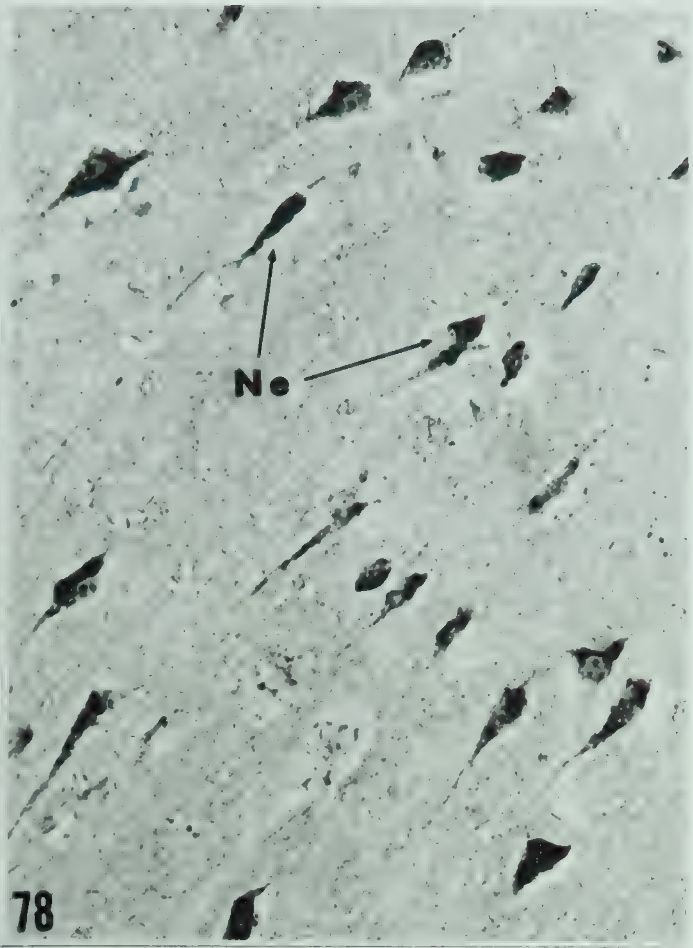

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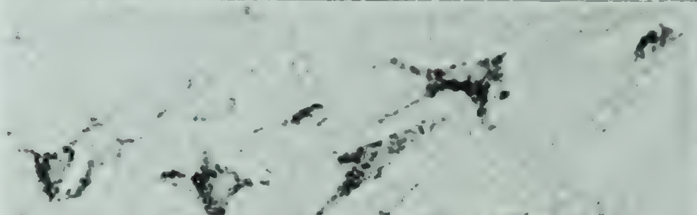

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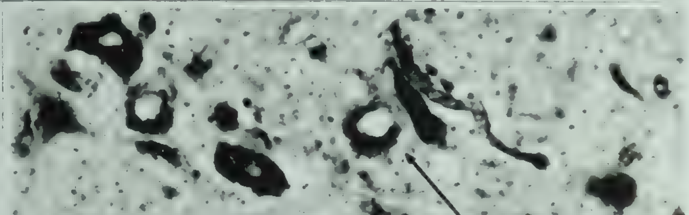

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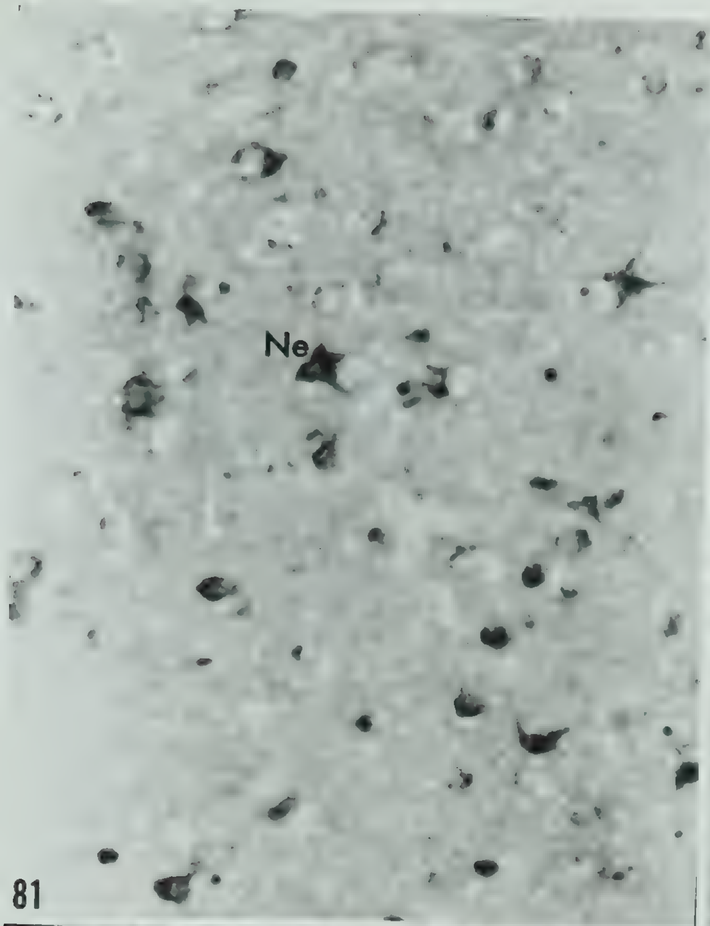
Fig. 81. Man (infant) brain, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 82. Chicken brain, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 83. Robin brain, B-glycerophosphate phosphatase. 250 X.

Fig. 84. Robin brain, Naphthol AS-acetate esterase. 250 X.



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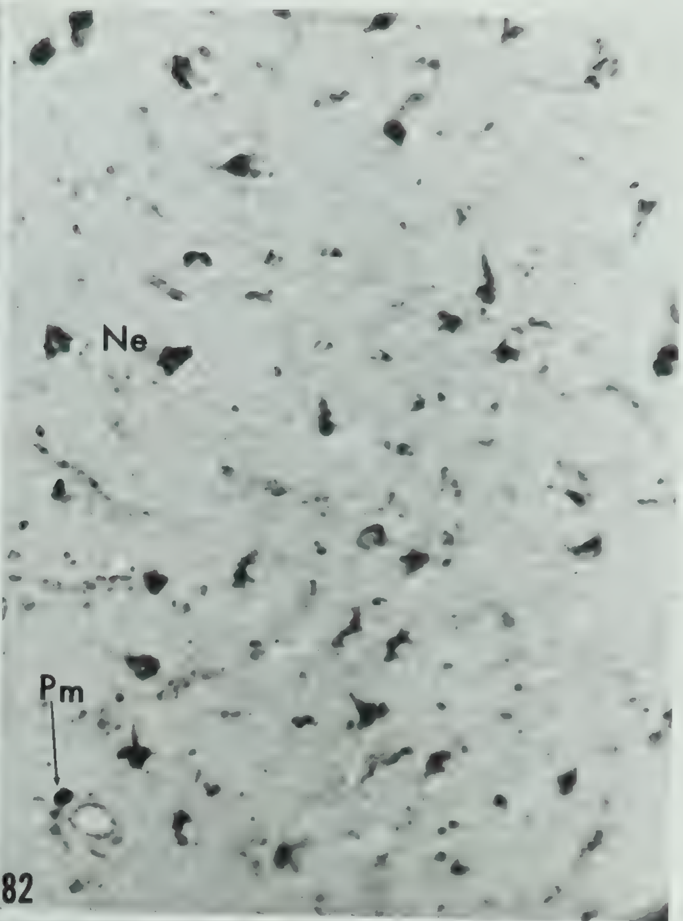

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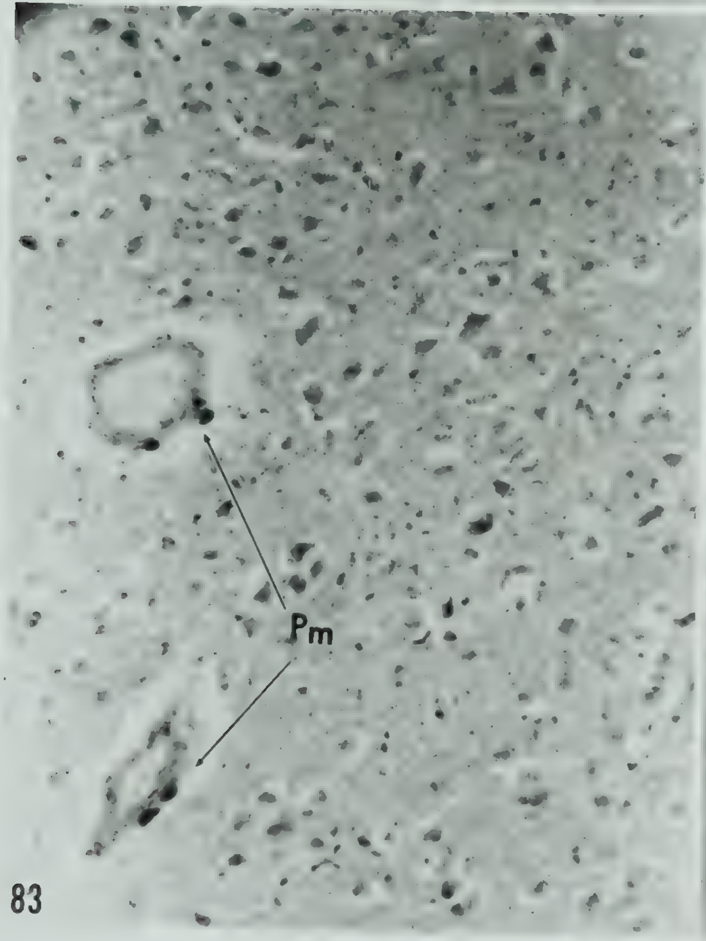

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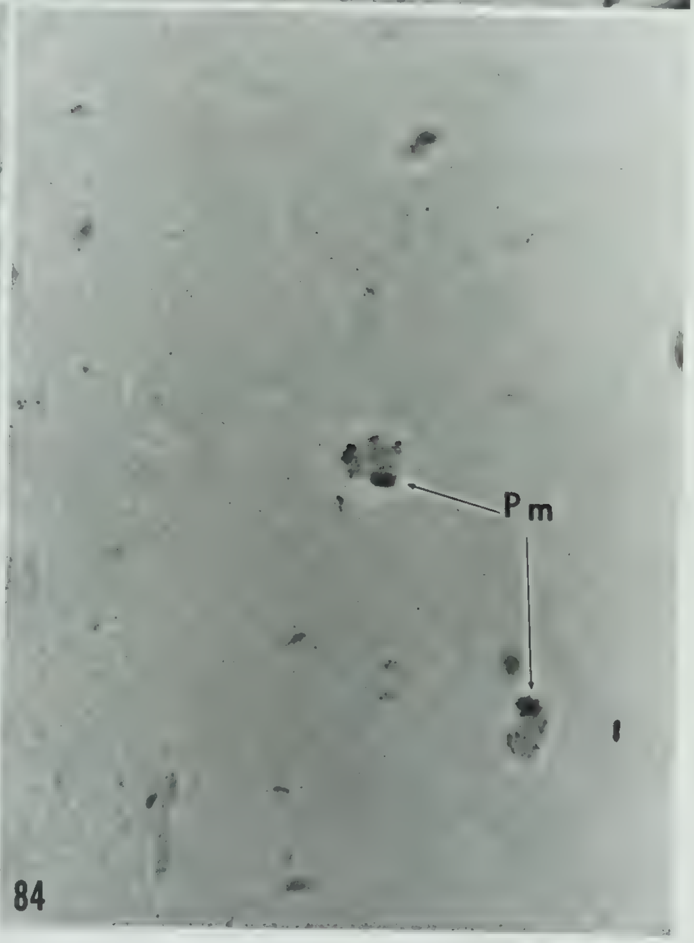

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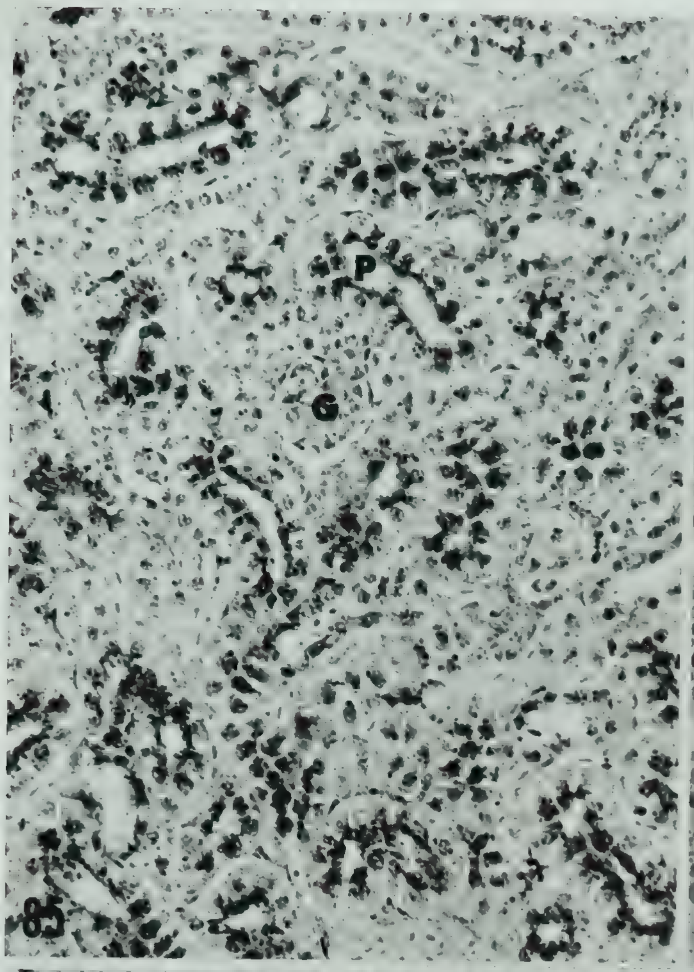
Fig. 85. Magpie kidney, B-glycerophosphate phosphatase, note the small glomerulus (G), and the intense enzyme activity in lysosomes of the proximal convoluted tubules (P). 250 X.

Fig. 86. Magpie kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 87. Magpie kidney, B-glucuronidase. 250 X.

Fig. 88. Japanese quail kidney, Naphthol AS-TR phosphate phosphatase. 250 X.



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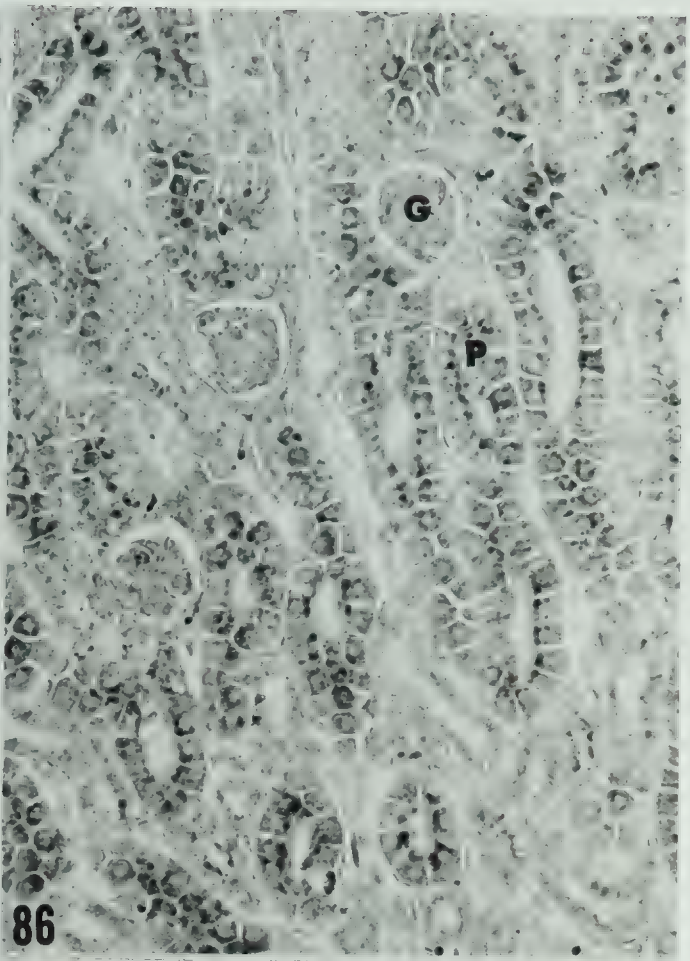

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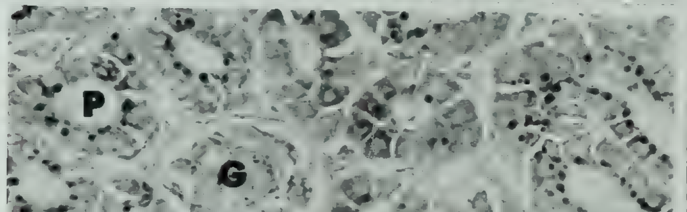

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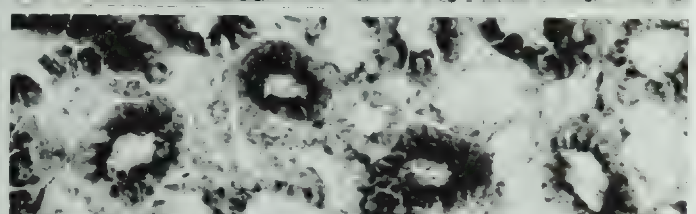

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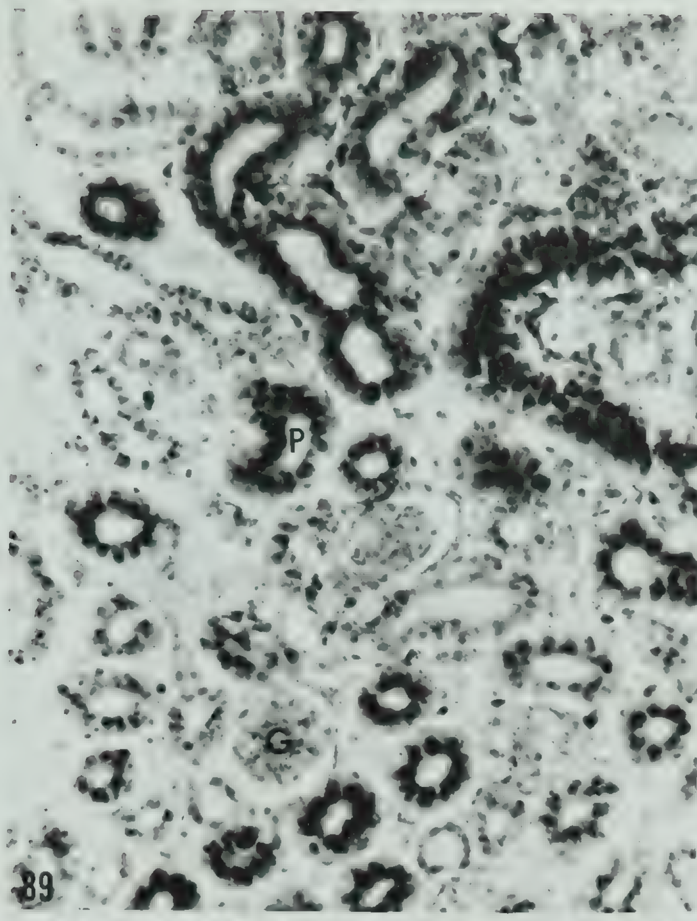
Fig. 89. Robin kidney, B-glycerophosphate phosphatase. 250 X.

Fig. 90. Chicken kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 91. Chicken liver, Naphthol AS-TR phosphate phosphatase, note the lysosomal enzyme activity in the Kupffer cells (K). 250 X.

Fig. 92. Robin liver, B-glycerophosphate phosphatase. 250 X.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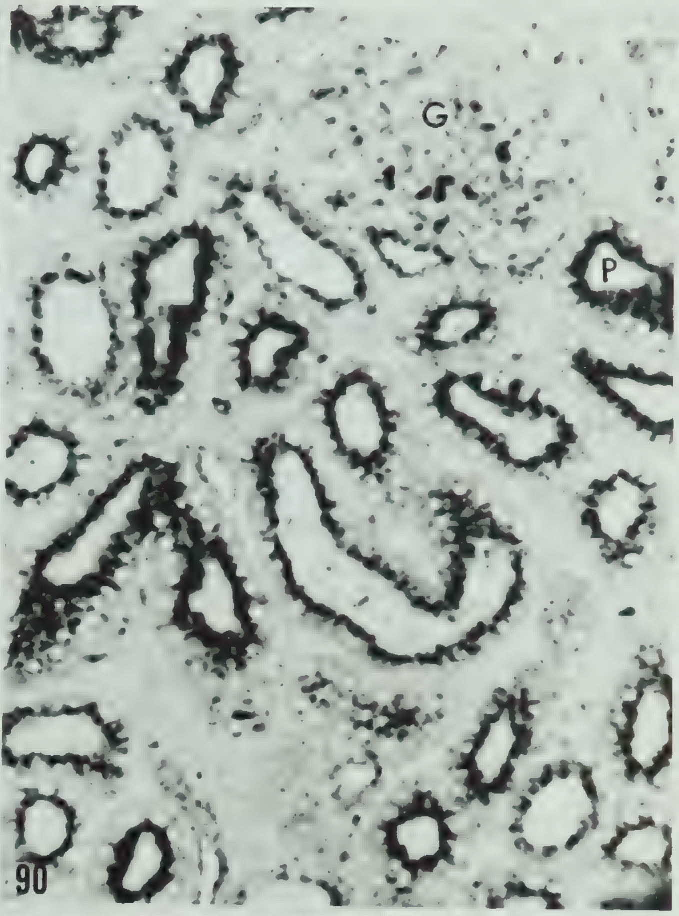
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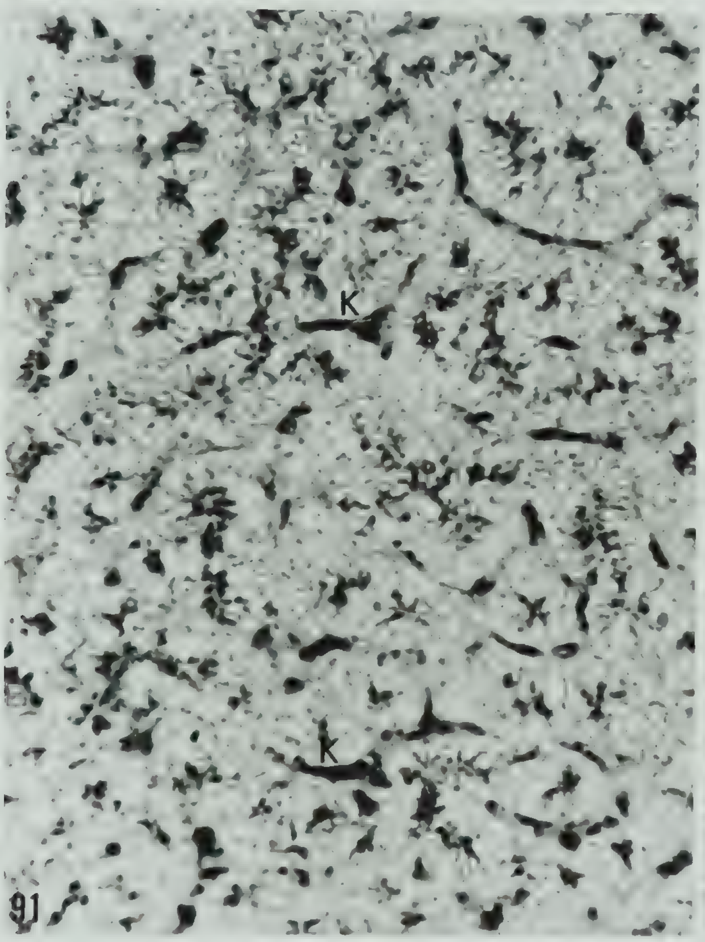
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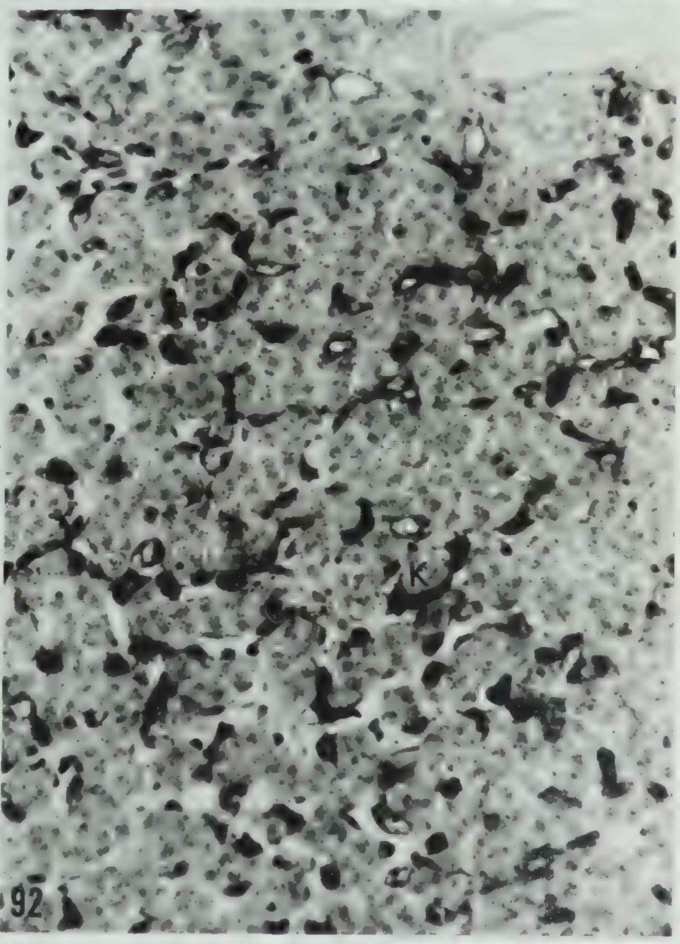
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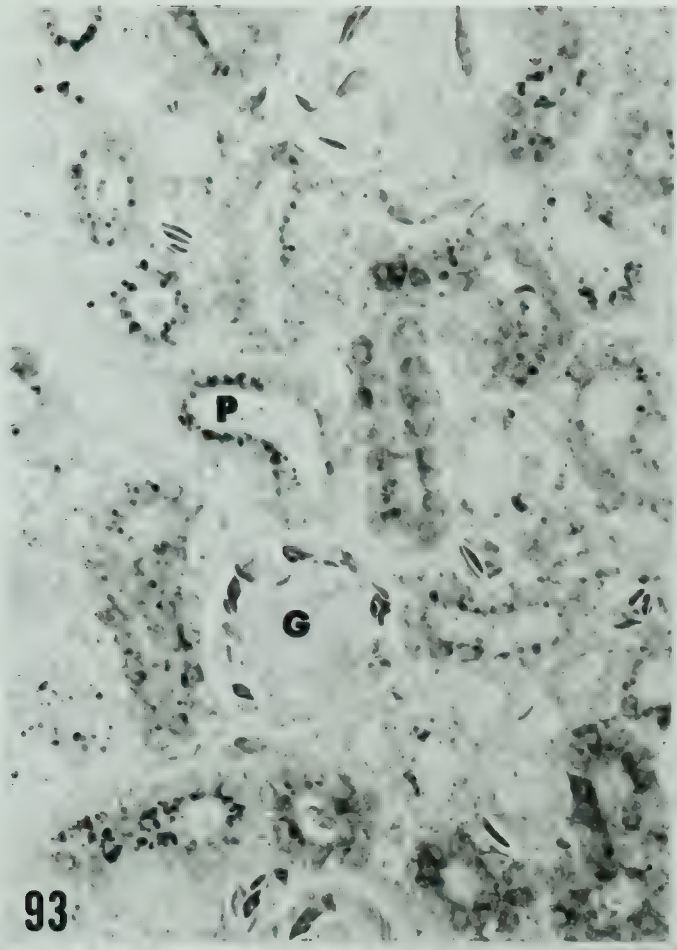
Fig. 93. Owl kidney, glucosaminidase. 250 X.

Fig. 94. Owl kidney, B-glycerophosphate phosphatase. 250 X.

Fig. 95. Magpie liver, B-glucuronidase. 250 X.

Fig. 96. Japanese quail liver, Naphthol AS-TR phosphate phosphatase. 250 X.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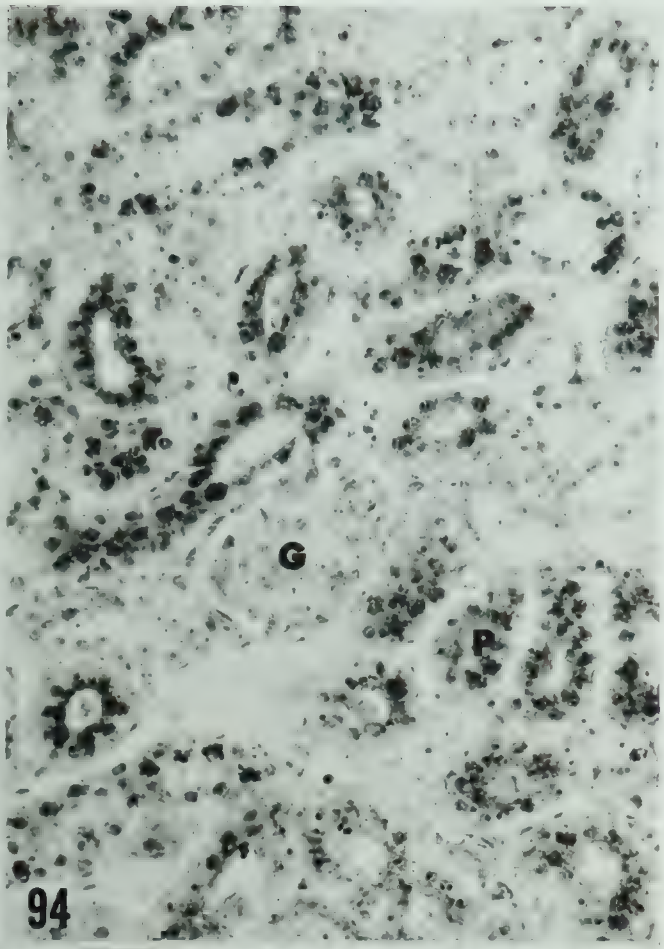
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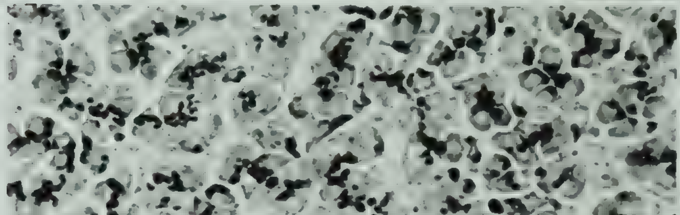
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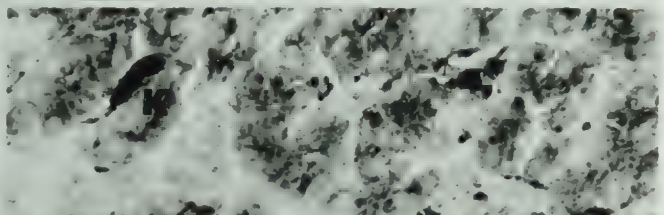
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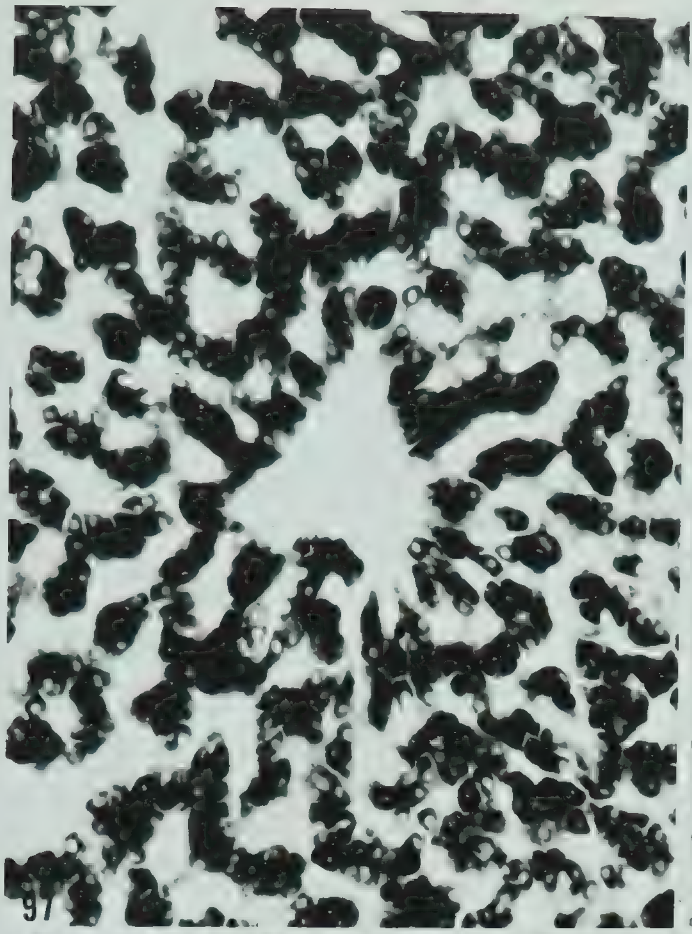
Fig. 97. Robin liver, a-Naphthyl acetate esterase. 250 X.

Fig. 98. Chicken spleen, B-glycerophosphate phosphatase. 250 X,

Fig. 99. Robin spleen, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 100. Robin spleen, a-Naphthyl acetate esterase. 250 X.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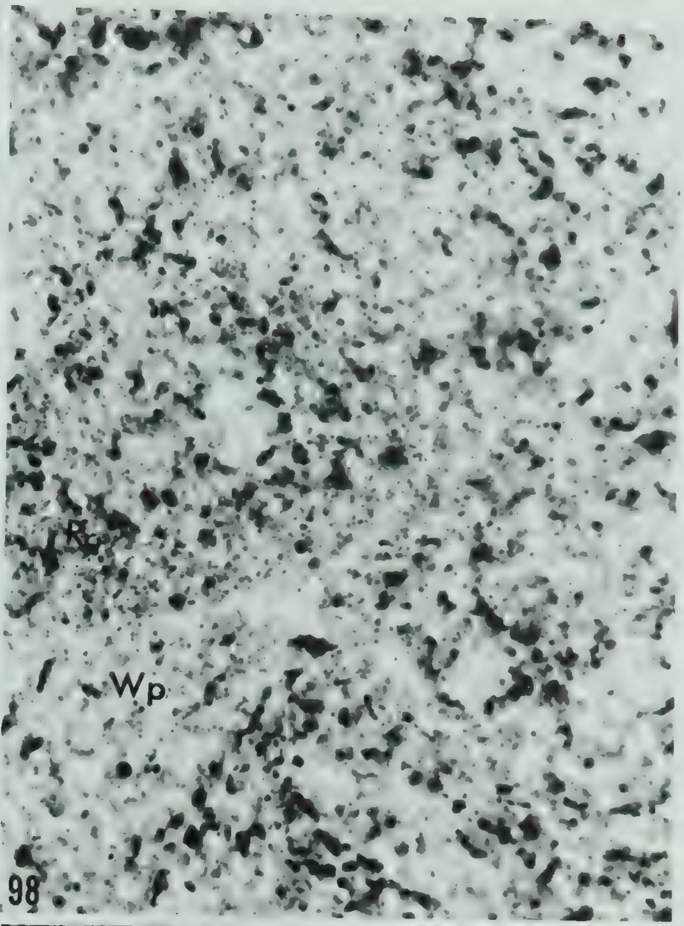
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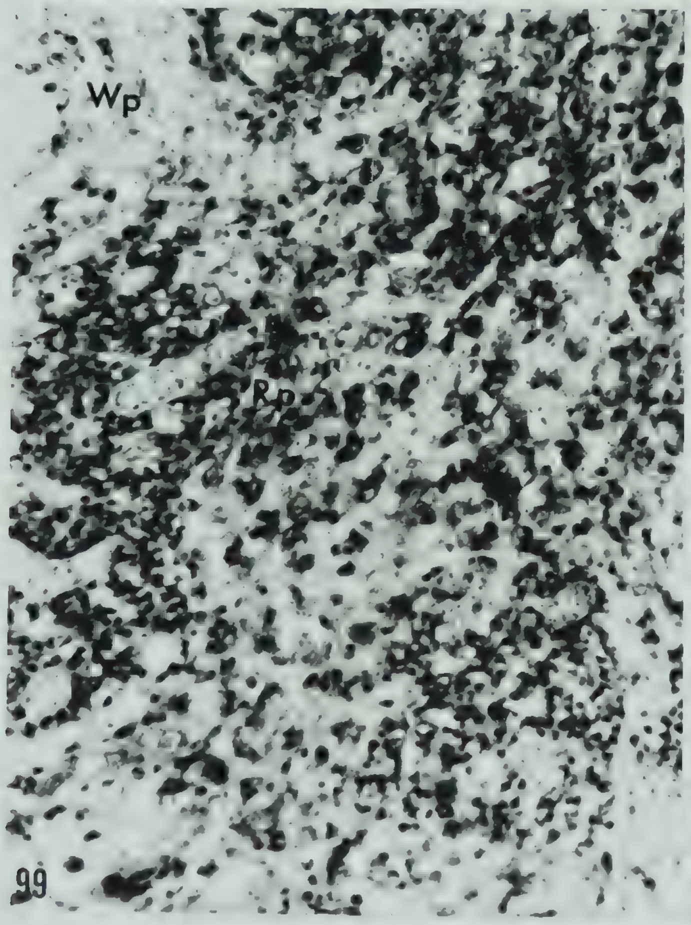
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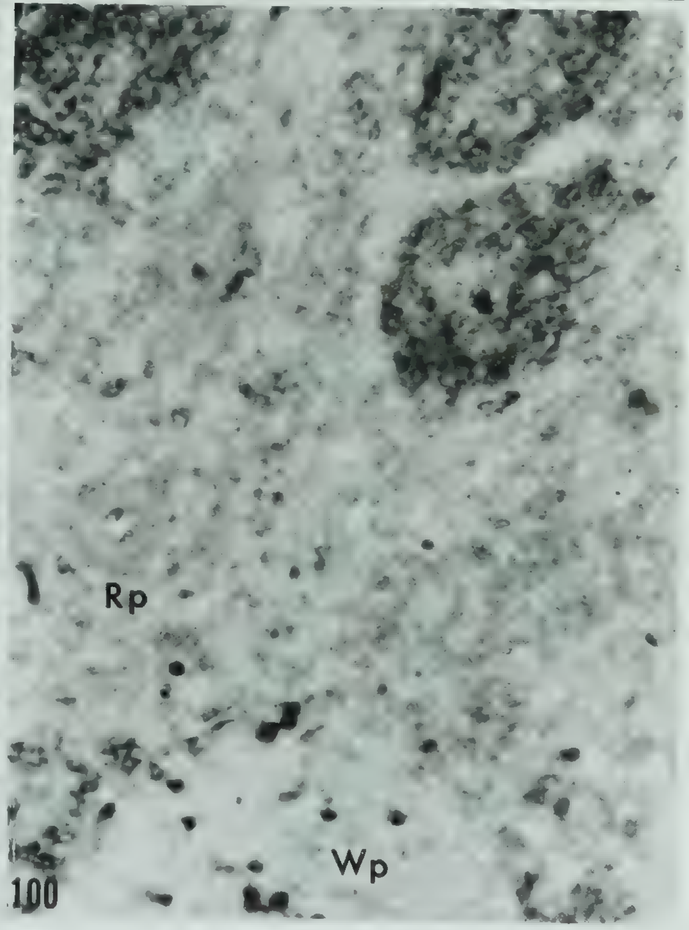
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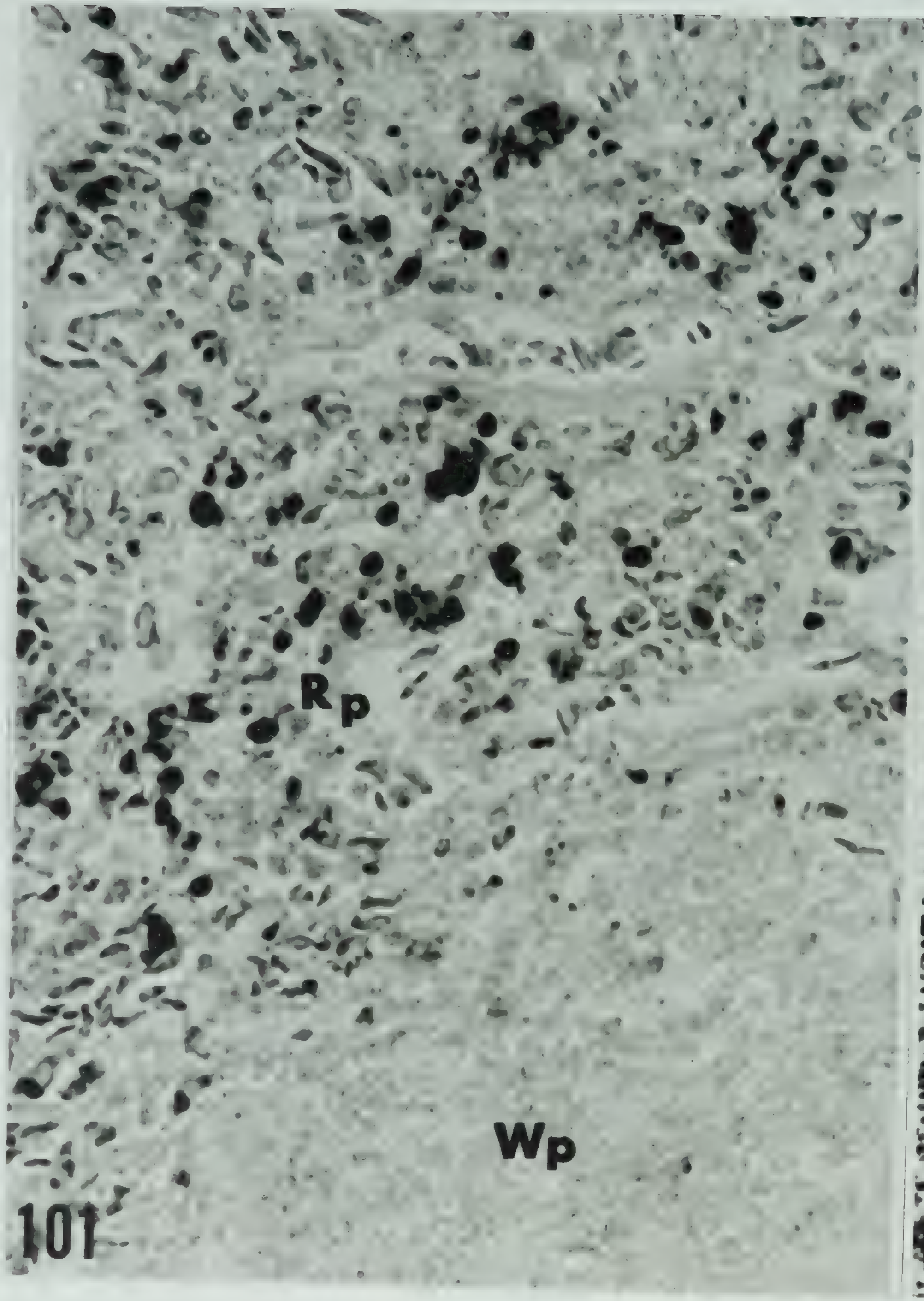
Fig. 101. Owl spleen, glucosaminidase, 376 X.

Fig. 102. Magpie spleen, B-glycerophosphate phosphatase. 376 X.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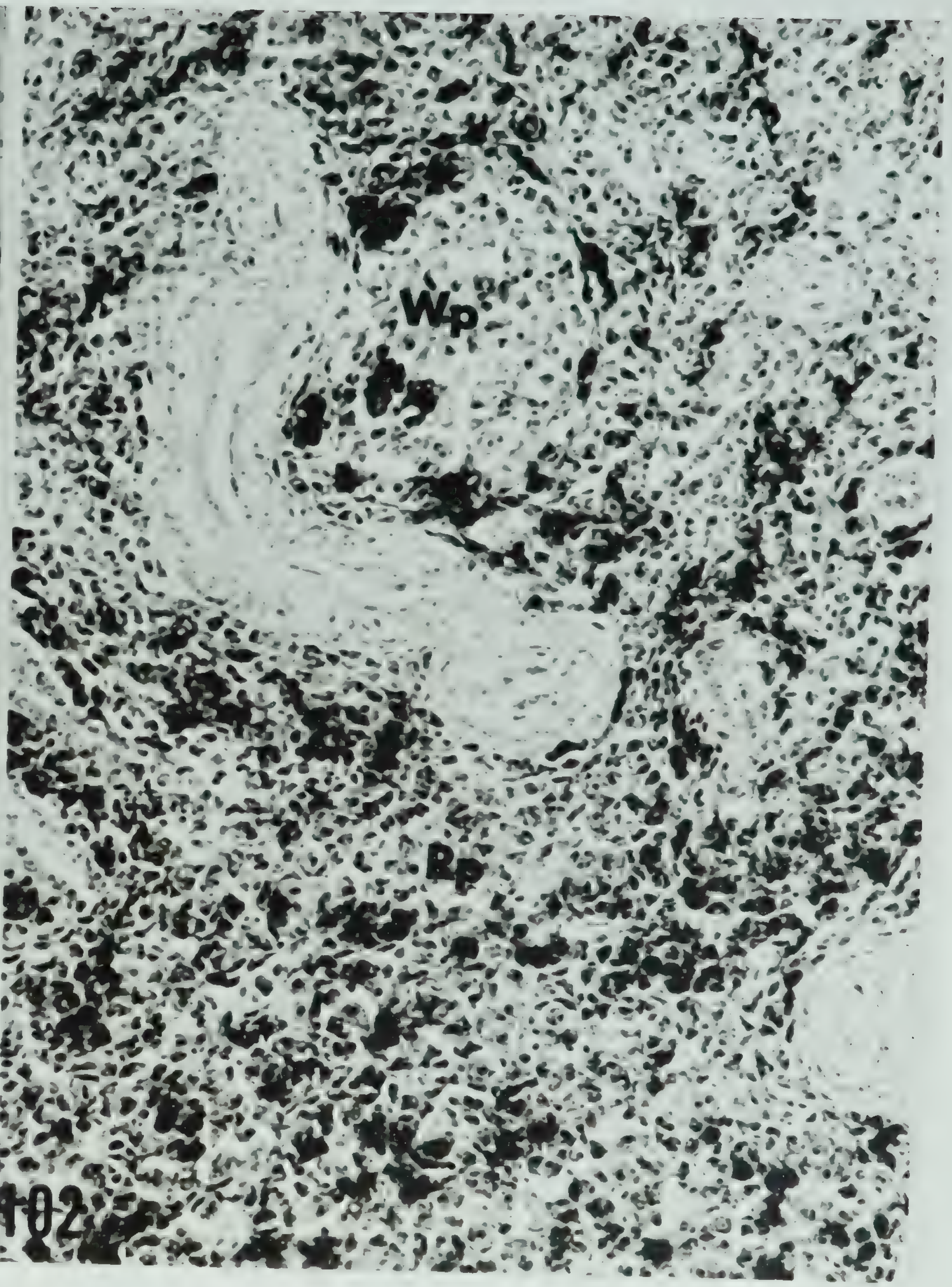
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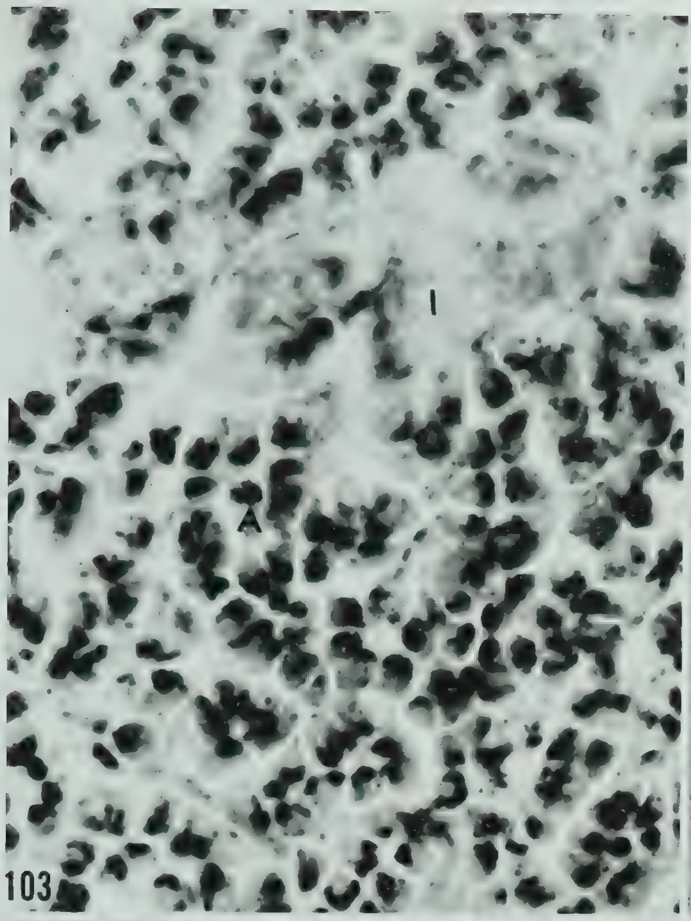
Fig. 103. Robin pancreas, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 104. Robin pancreas, B-glucuronidase, note the enzyme activity confined to the Islet of Langerhans (I). 250 X.

Fig. 105. Chicken pancreas, B-glycerophosphate phosphatase. 250 X.

Fig. 106. Robin duodenum, B-glycerophosphate phosphatase. 250 X.



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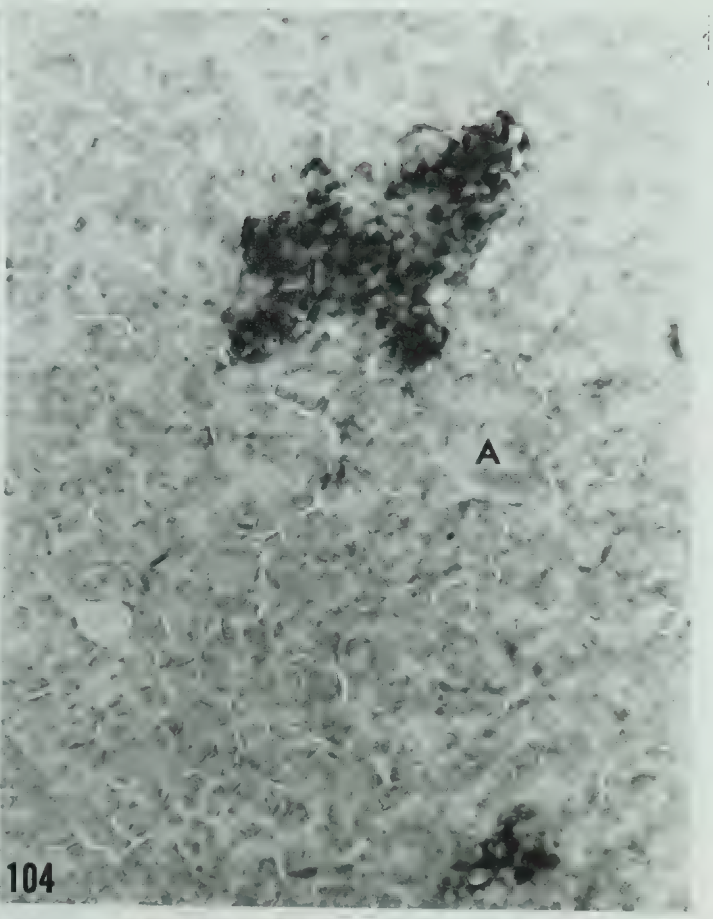

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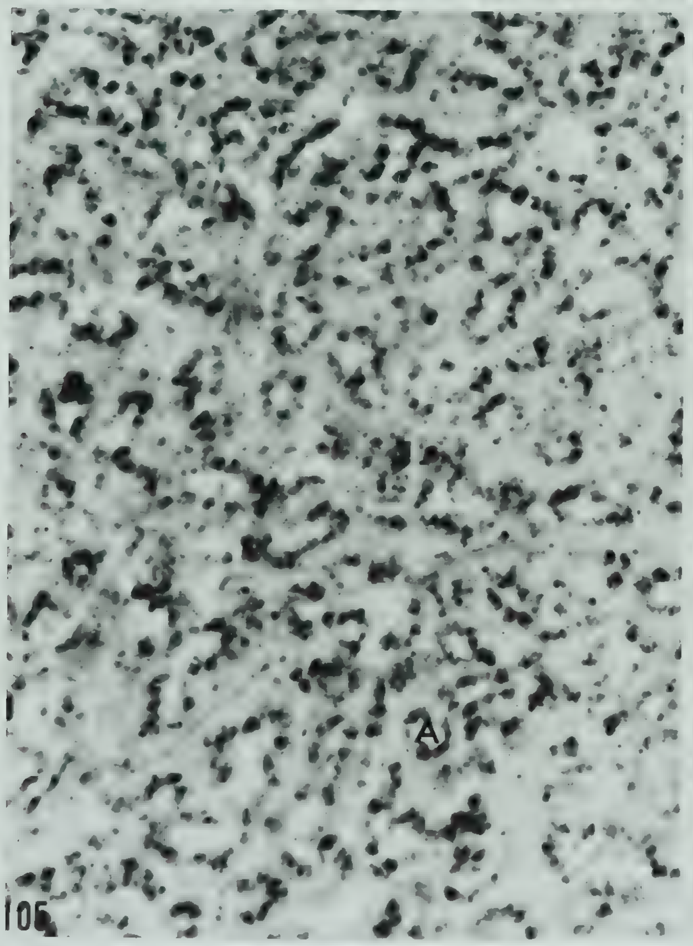

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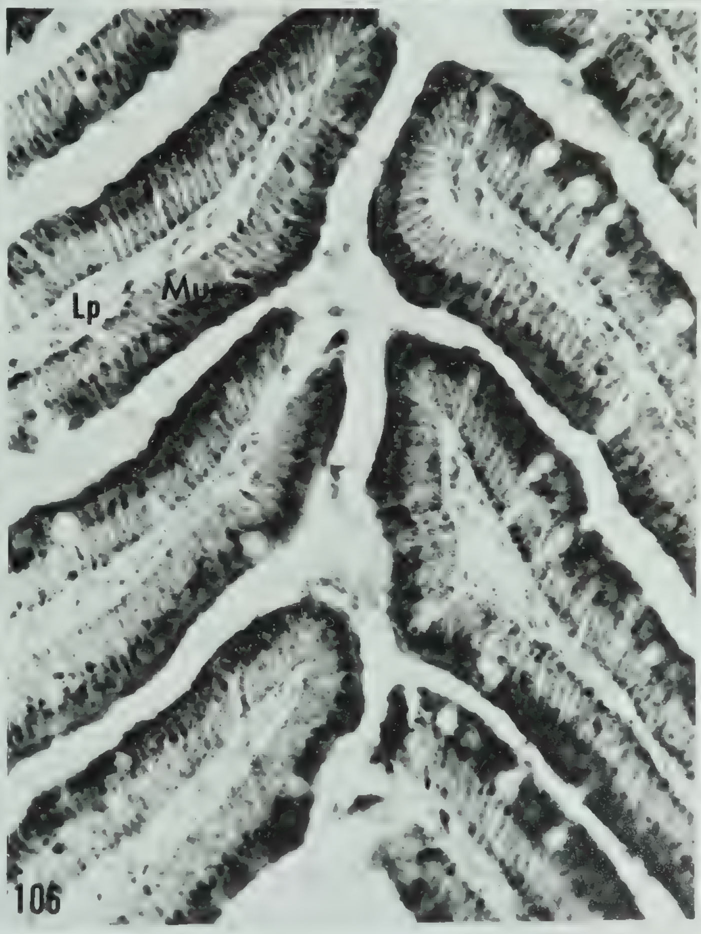

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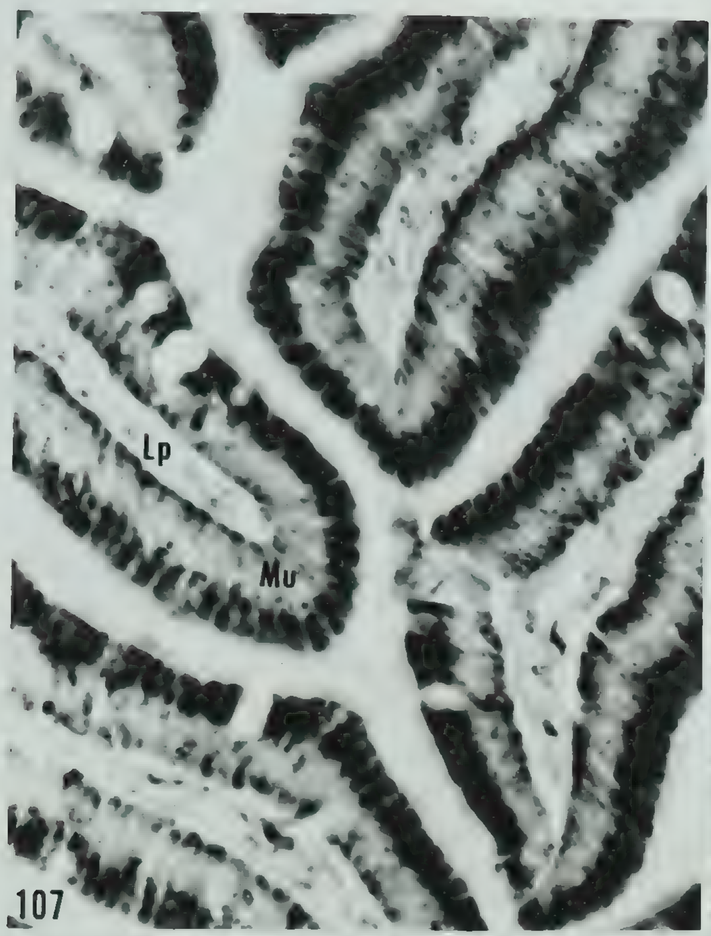
Fig. 107. Robin duodenum, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 108. Robin duodenum, B-glucuronidase. 250 X.

Fig. 109. Robin duodenum, glucosaminidase. 250 X.

Fig. 110. Robin duodenum, a-Naphthyl acetate esterase. 250 X.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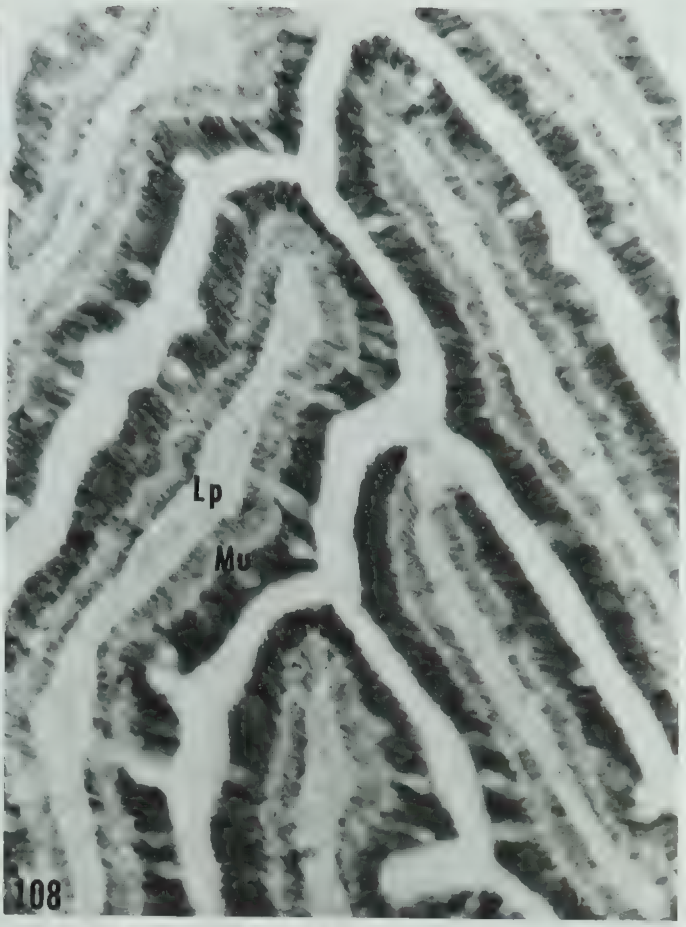
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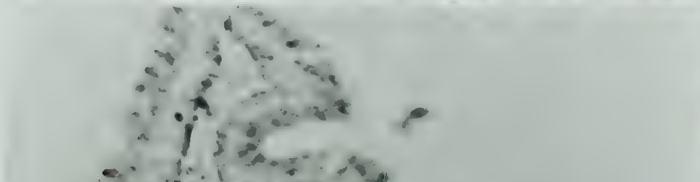
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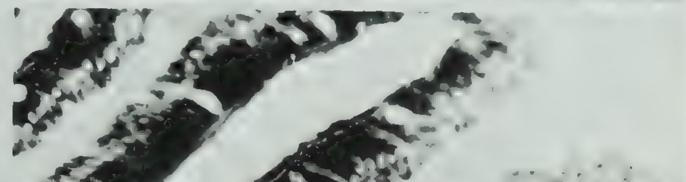
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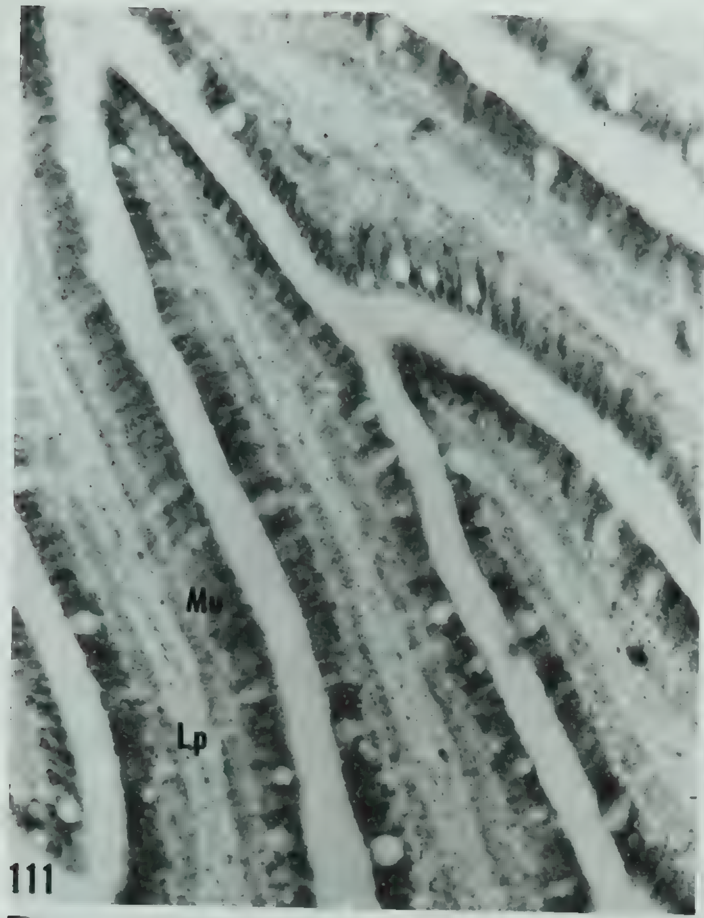
Fig. 111. Robin duodenum, Naphthol AS acetate esterase. 250 X.

Fig. 112. Chicken duodenum, B-glycerophosphate phosphatase. 250 X.

Fig. 113. Chicken duodenum, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 114. Chicken duodenum, Naphthol AS acetate esterase. 250 X.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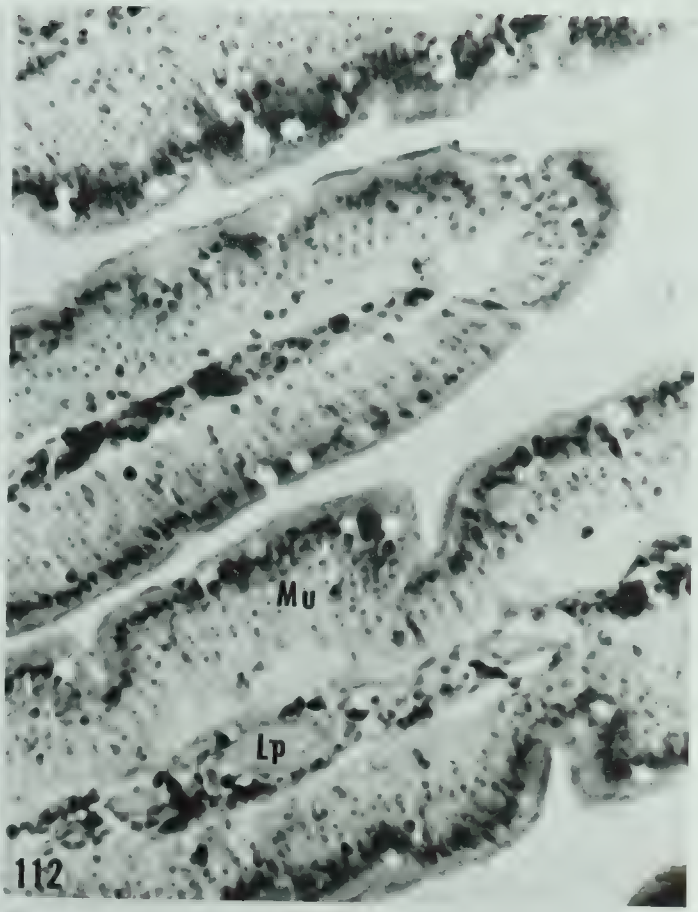
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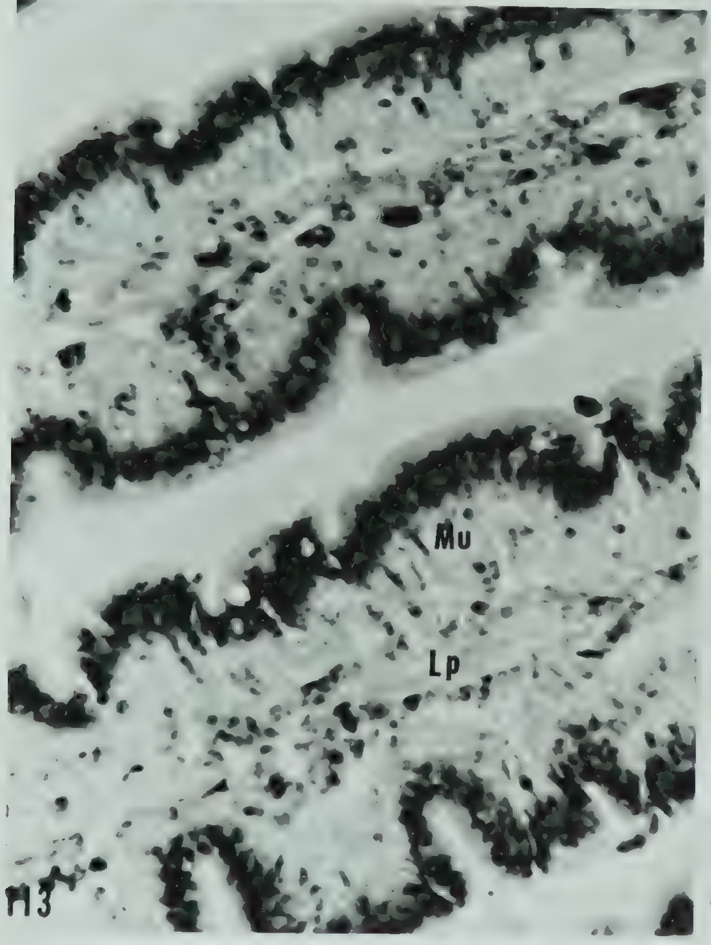
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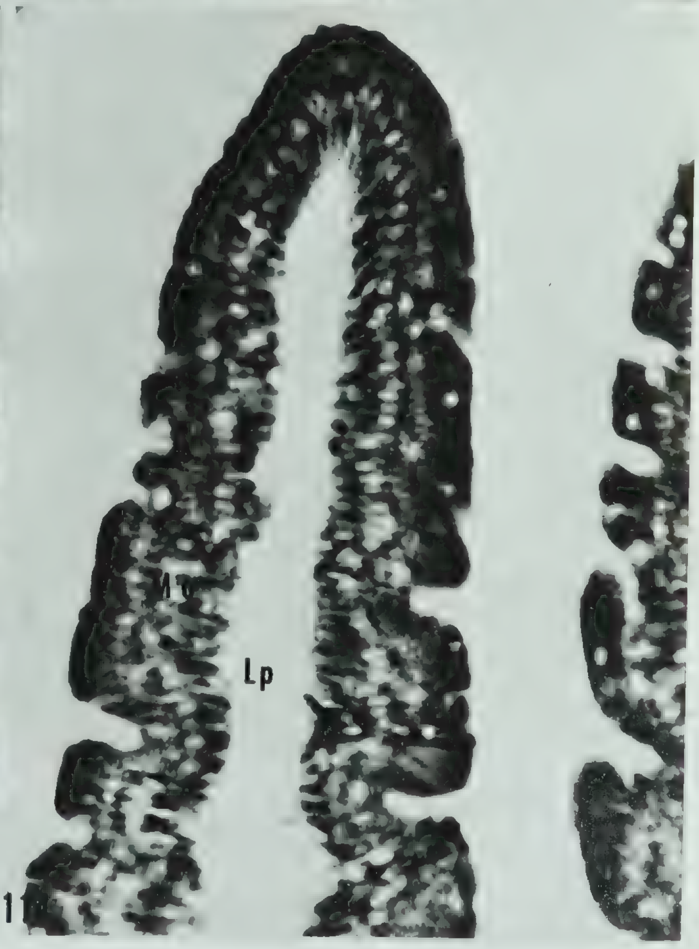
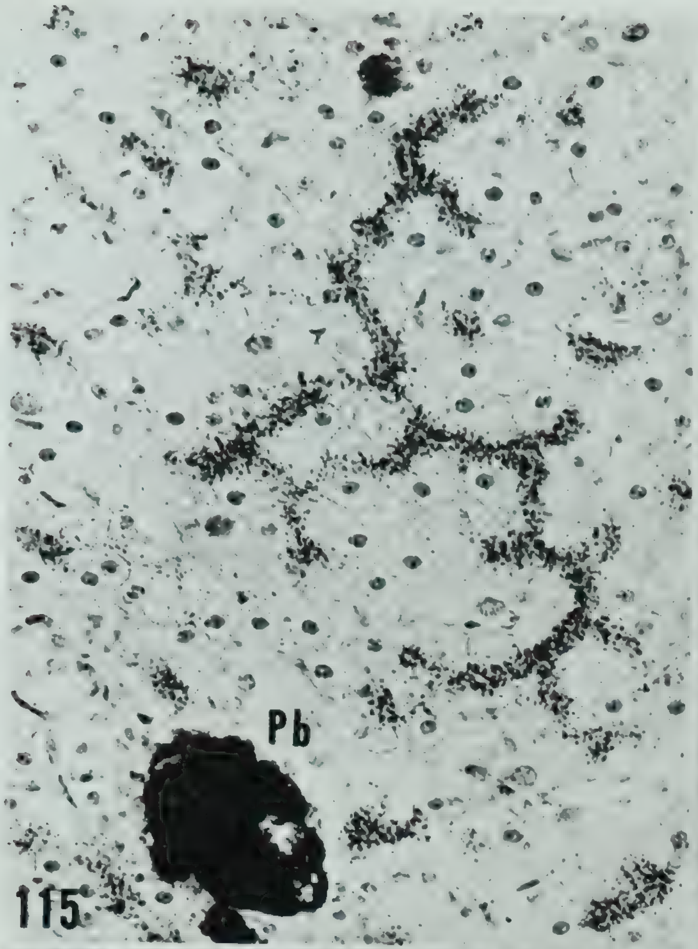

114

Fig. 115. Turtle liver, B-glycerophosphate phosphatase, note large pigment body (Pb) and intense lysosomal enzyme activity in the pericanalicular region of parenchymal cells. 250 X.

Fig. 116. Turtle liver, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 117. Turtle liver, B-glucuronidase. 250 X.

Fig. 118. Turtle liver, glucosaminidase. 250 X.



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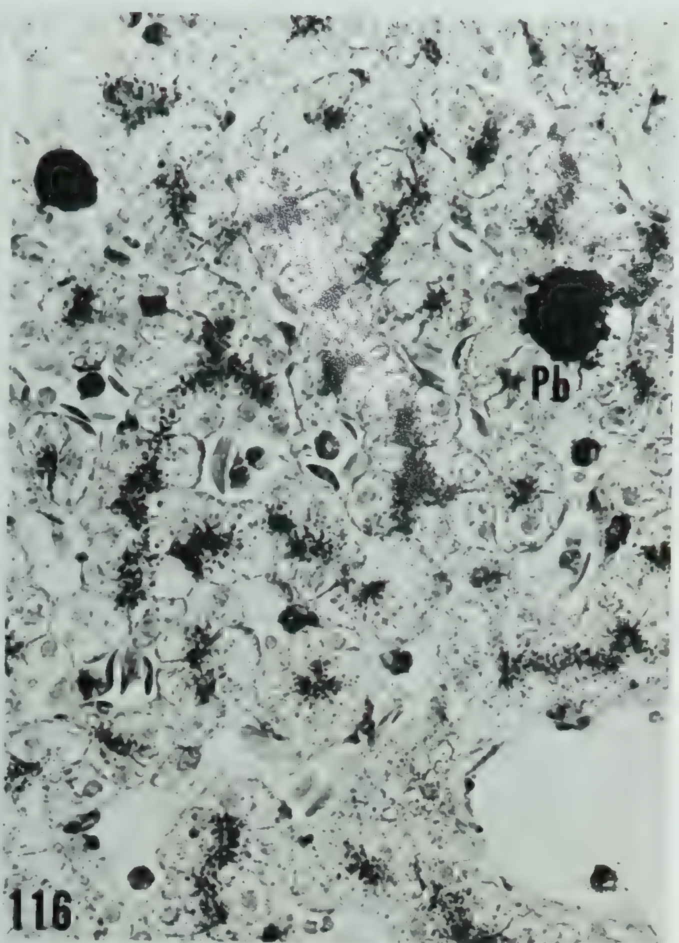

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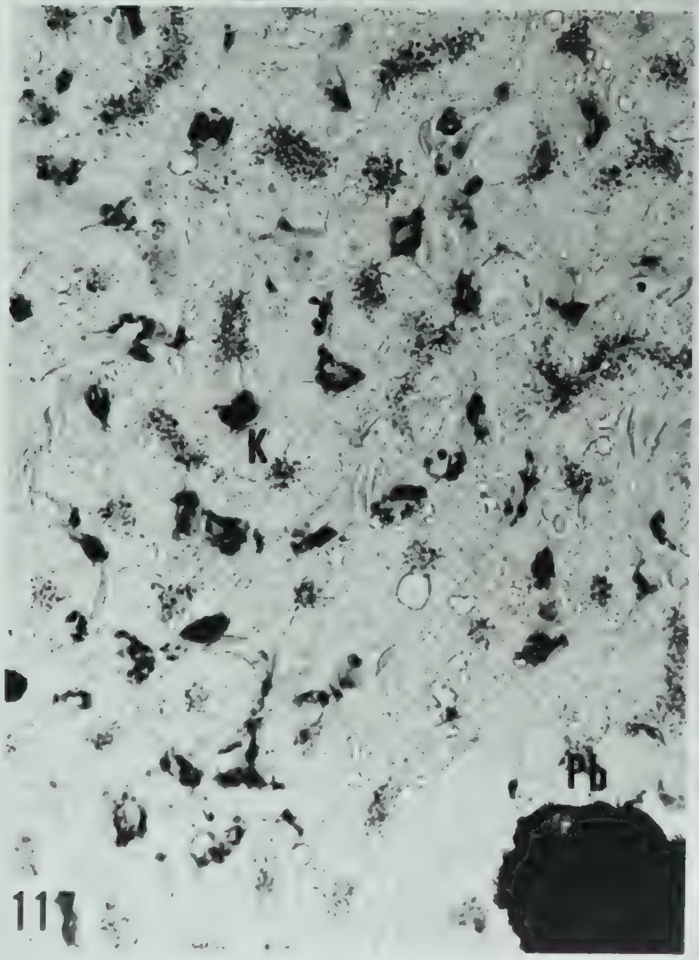

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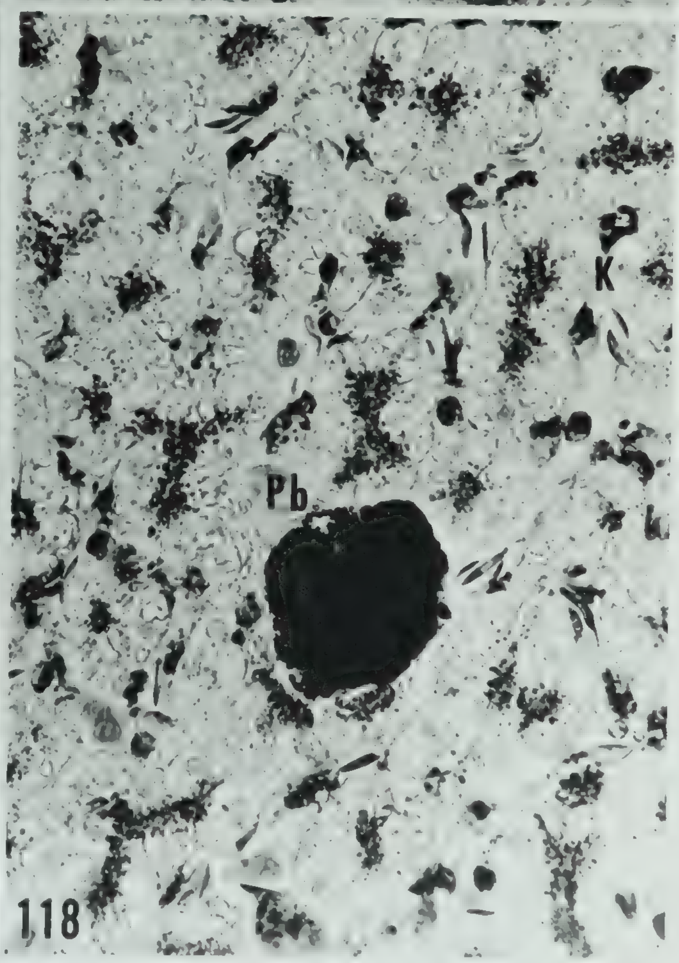

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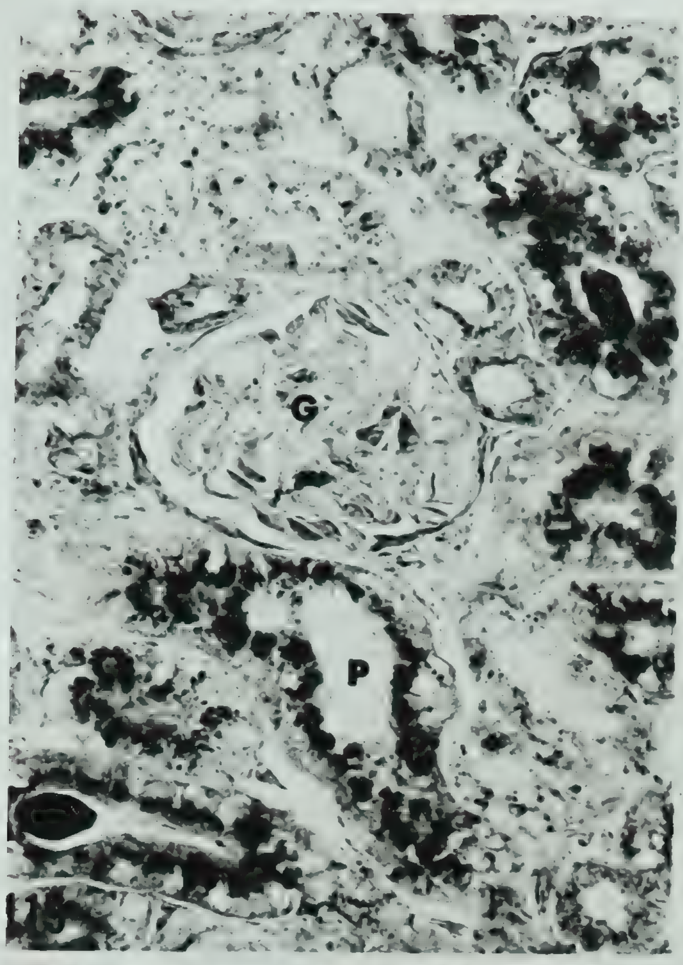
Fig. 119. Turtle kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 120. Turtle kidney, glucosaminidase. 250 X.

Fig. 121. Turtle kidney, a-Naphthyl acetate esterase. 250 X.

Fig. 122. Turtle pancreas, a-Naphthyl acetate esterase. 250 X.



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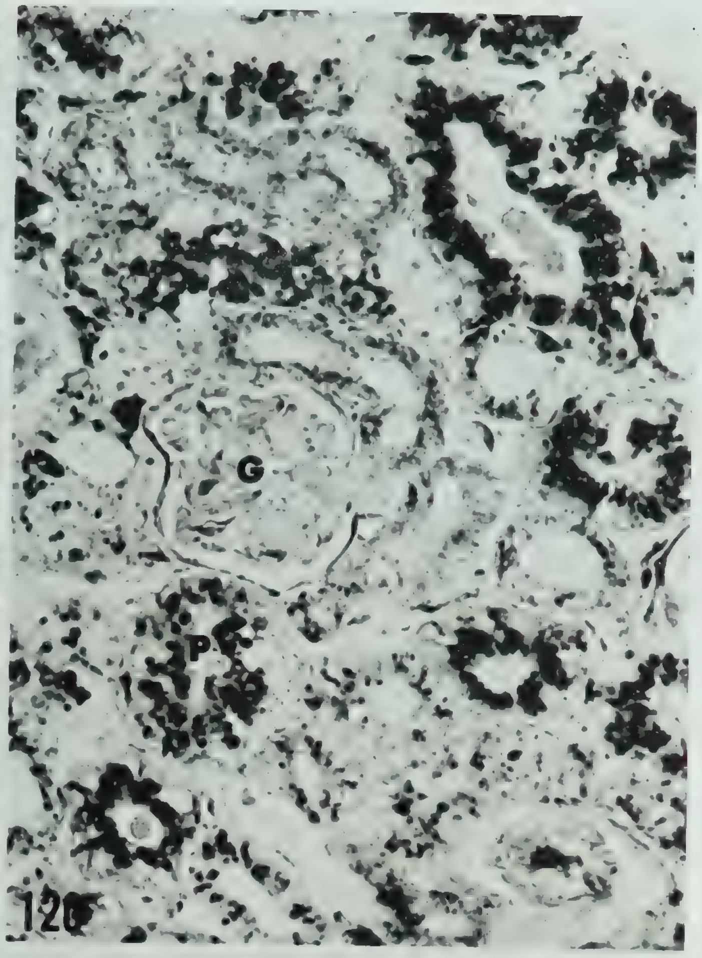

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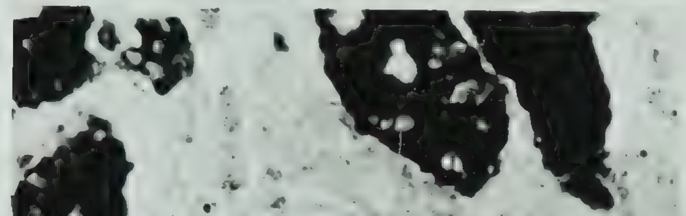

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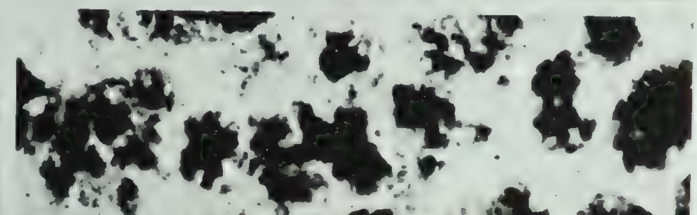

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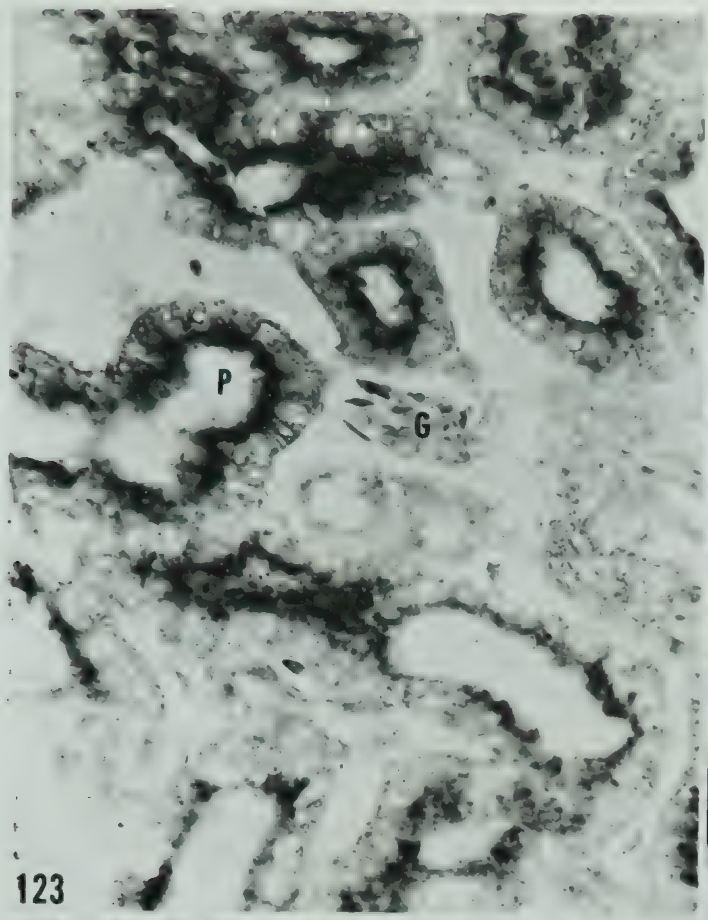
Fig. 123. Chameleon kidney, Naphthol AS-TR phosphate phosphatase, note the small glomerulus (G) and intense enzyme activity in the lysosomes of the proximal convoluted tubules (P). 250 X.

Fig. 124. Chameleon kidney, a-Naphthyl acetate esterase. 250 X.

Fig. 125. Chameleon liver, B-glycerophosphate phosphatase. 250 X.

Fig. 126. Chameleon liver, a-Naphthyl acetate esterase. 250 X.



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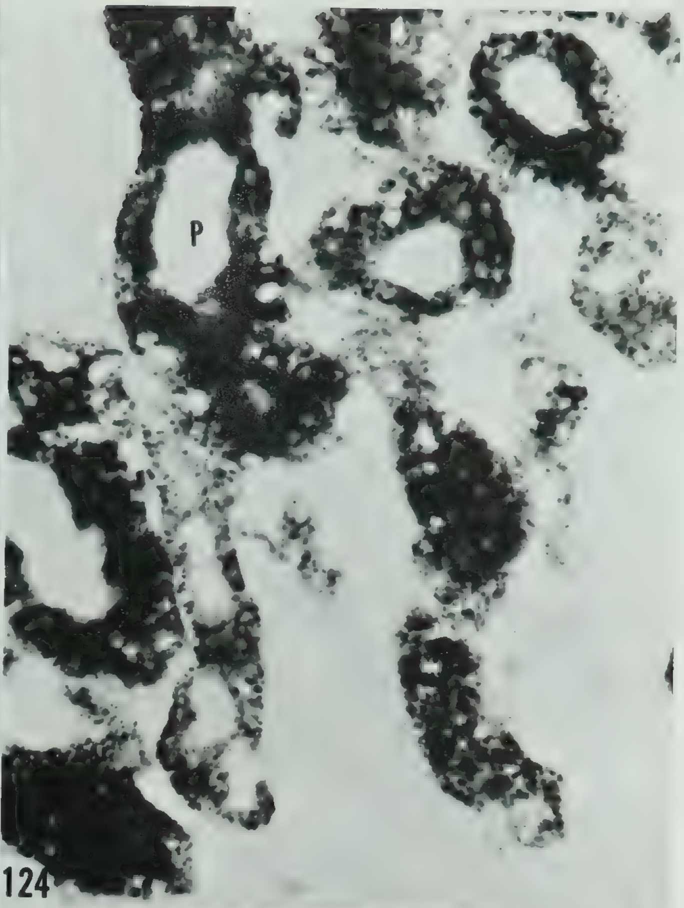

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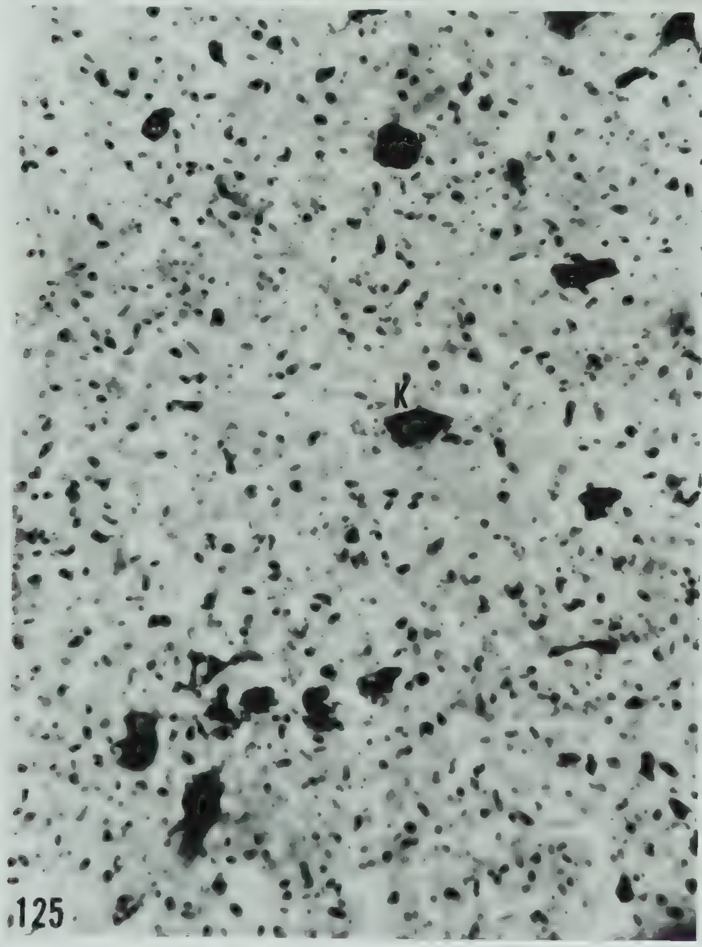

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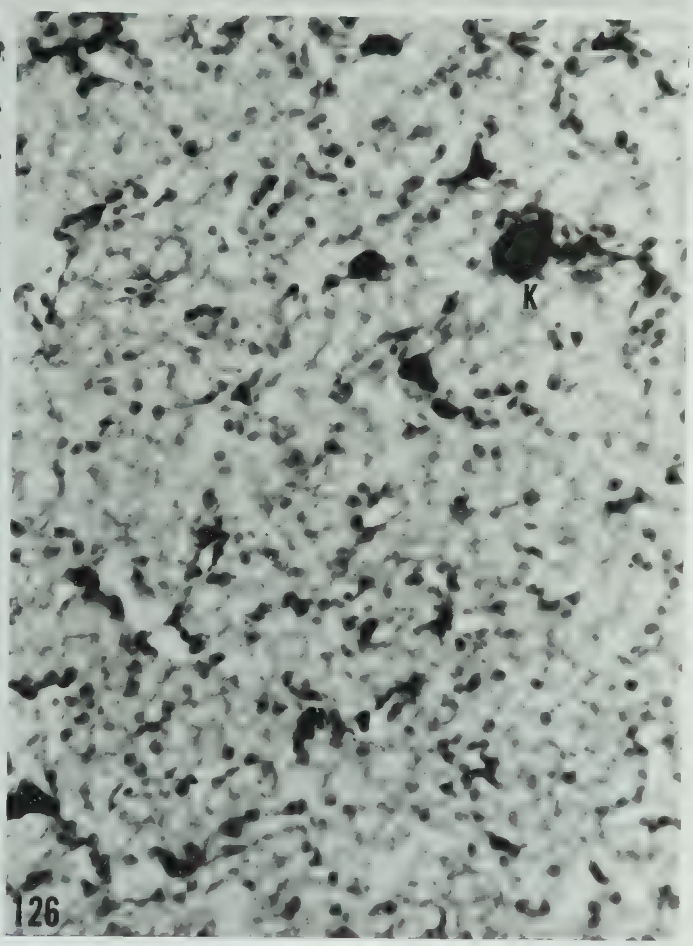

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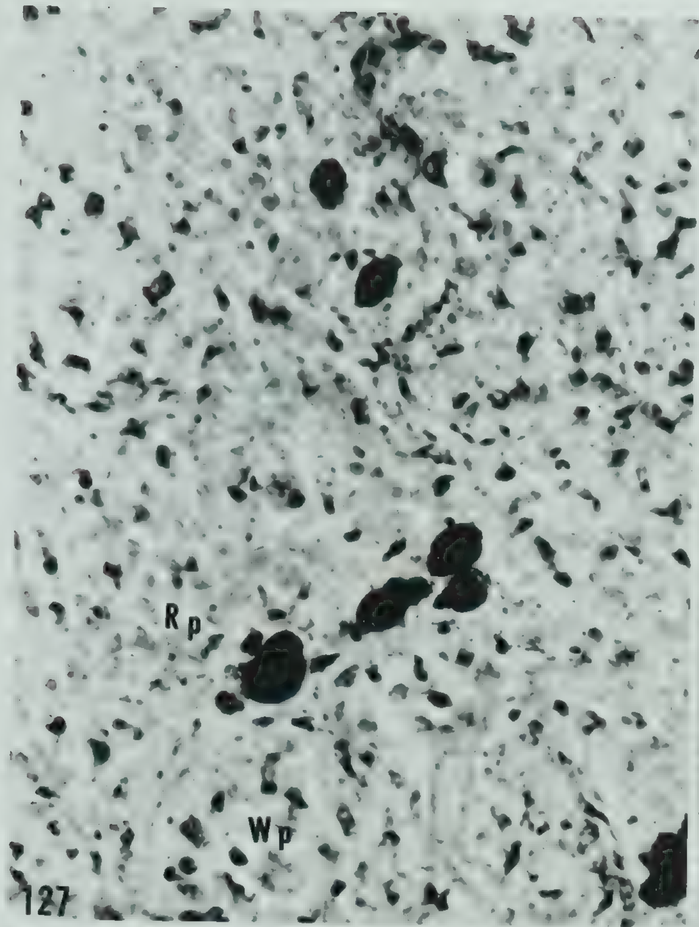
Fig. 127. Turtle spleen, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 128. Turtle spleen, B-glucuronidase. 250 X.

Fig. 129. Turtle spleen, glucosaminidase. 250 X.

Fig. 130. Turtle spleen, a-Naphthyl acetate esterase. 250 X.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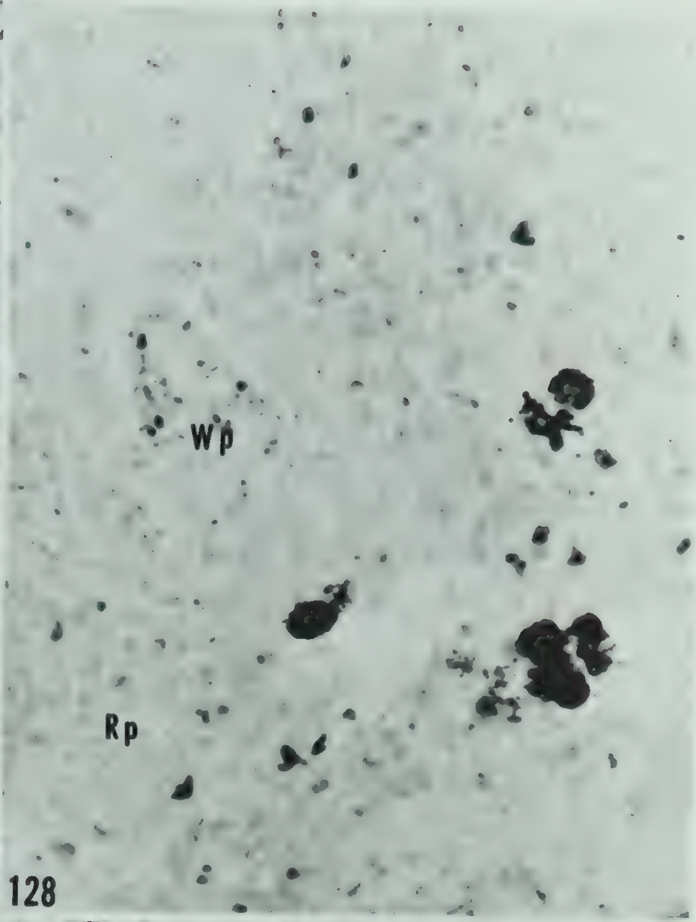
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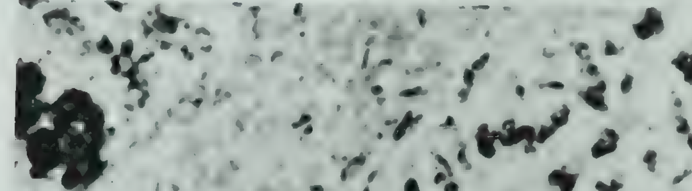
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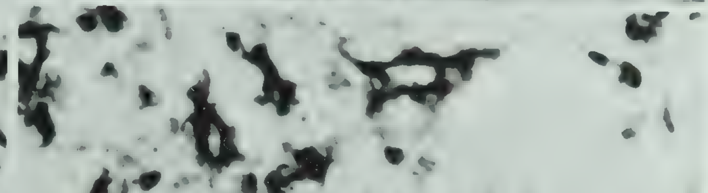
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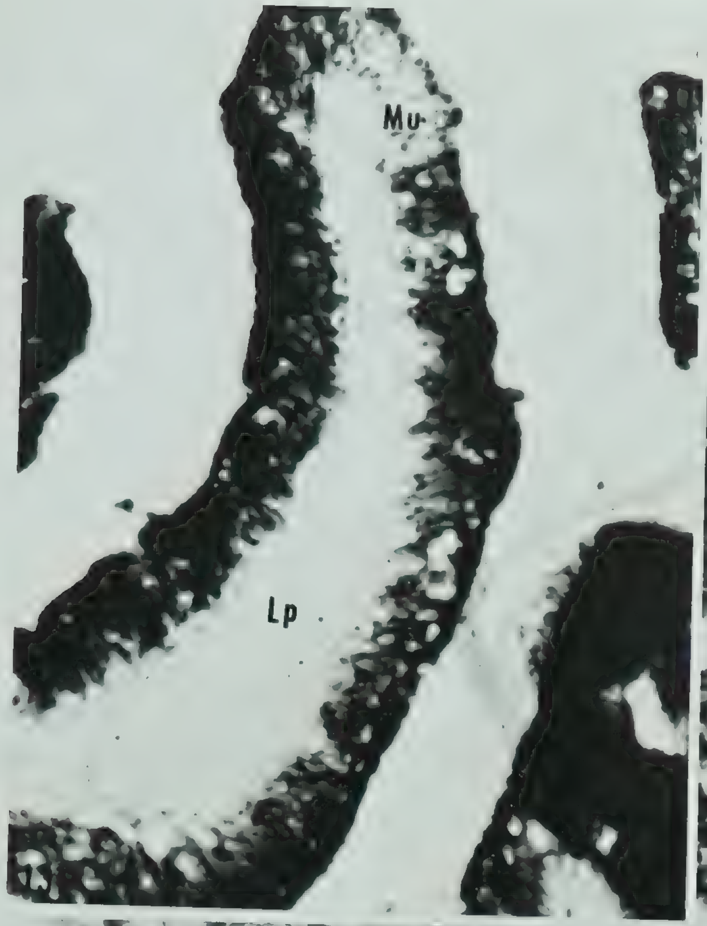
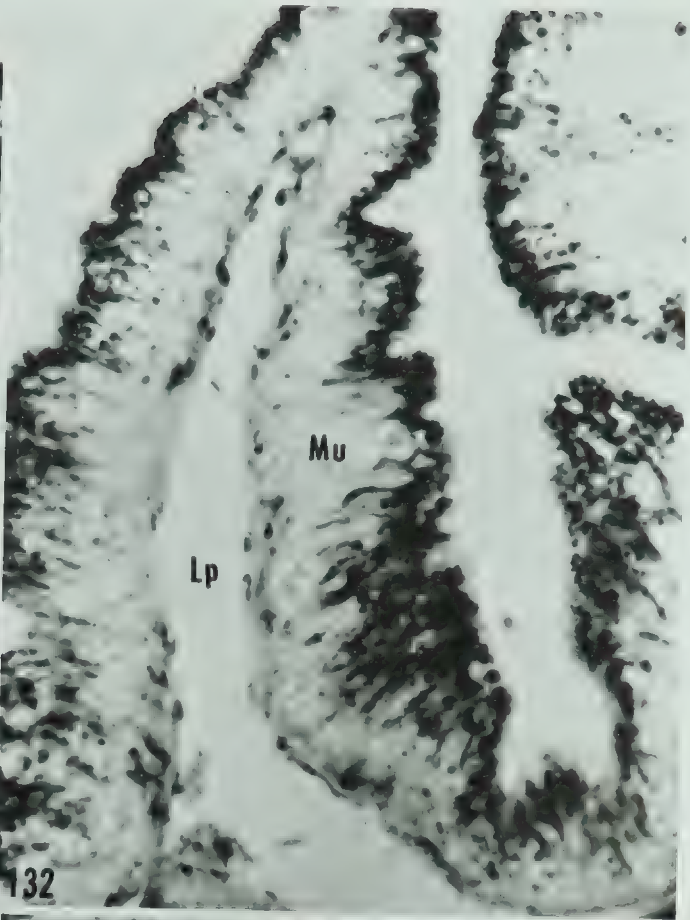
Fig. 131. Chameleon duodenum, a-Naphthyl acetate esterase. 250 X.

Fig. 132. Chameleon duodenum, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 133. Salamander kidney, B-glycerophosphate phosphatase. 250 X.

Fig. 134. Salamander kidney, glucosaminidase, note slight enzyme activity in the renal tubules (D) only, activity for this enzyme is not present in the red blood cells (rbc). 250 X.





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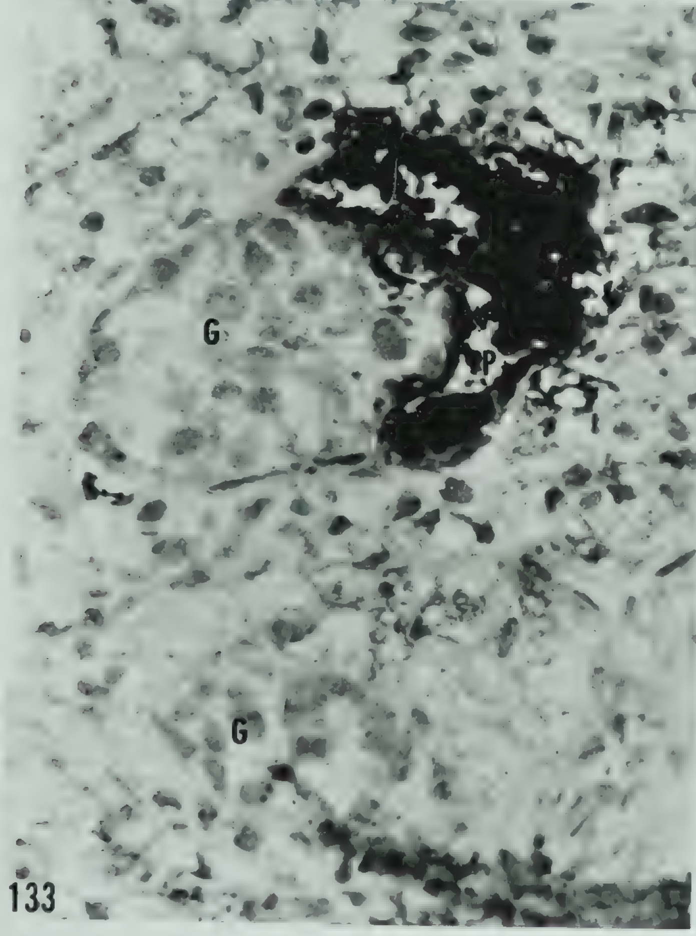

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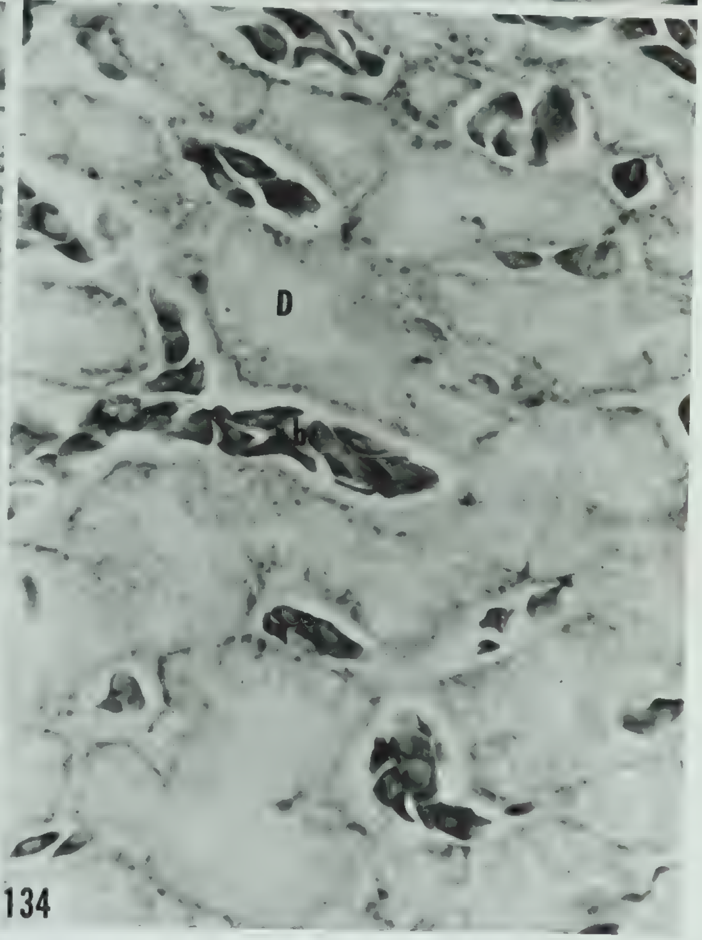

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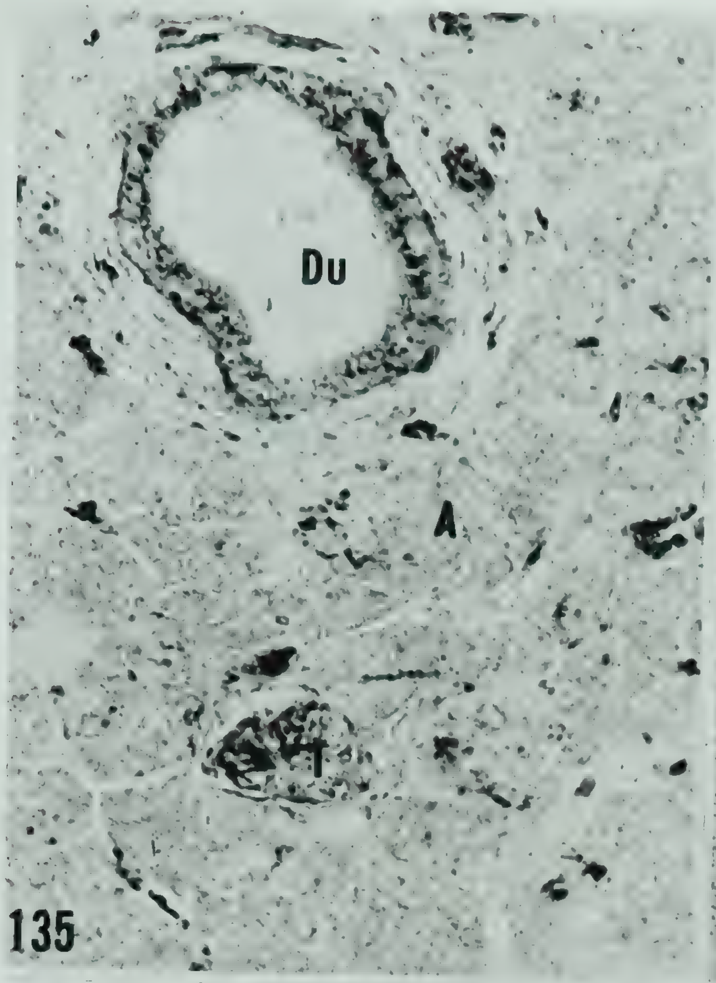
Fig. 135. Turtle pancreas, glucosaminidase, note the enzyme activity in the duct epithelium (D) and Islet of Langerhans (I). 250 X.

Fig. 136. Turtle brain, Naphthol AS-TR phosphate phosphatase, note intense lysosomal enzyme activity in the neurons (Ne). 250 X.

Fig. 137. Turtle brain, glucosaminidase. 250 X.

Fig. 138. Tree frog kidney, Naphthol AS-TR phosphate phosphatase. 250 X.



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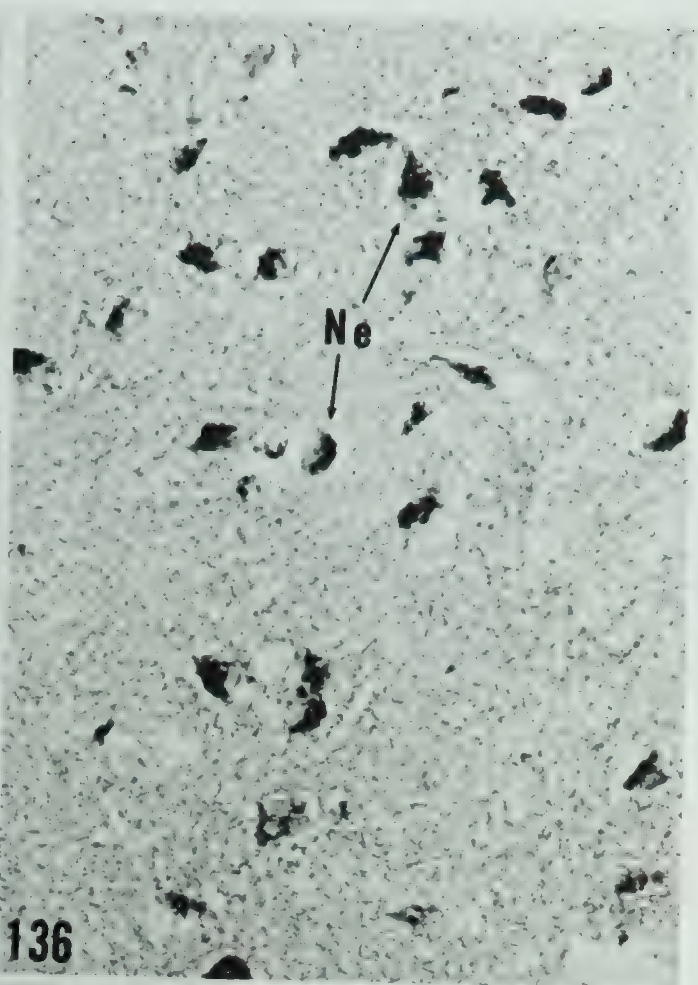

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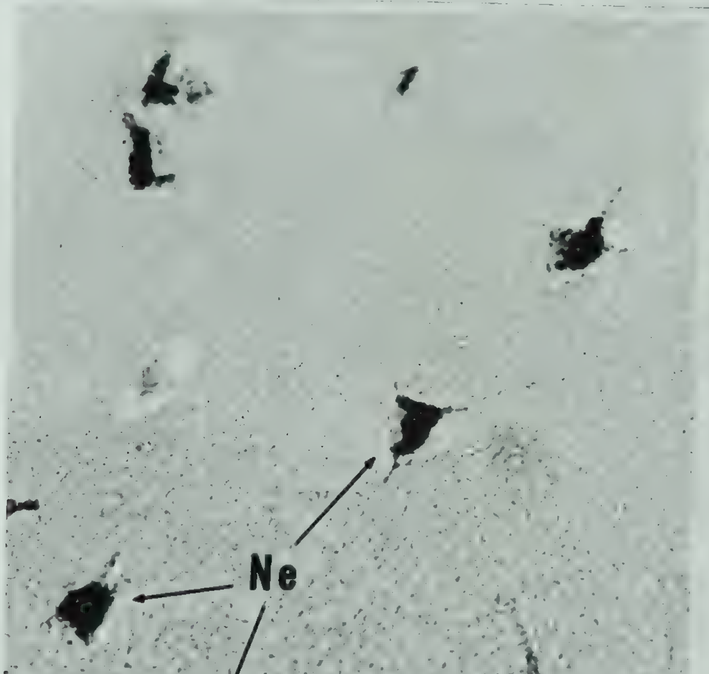

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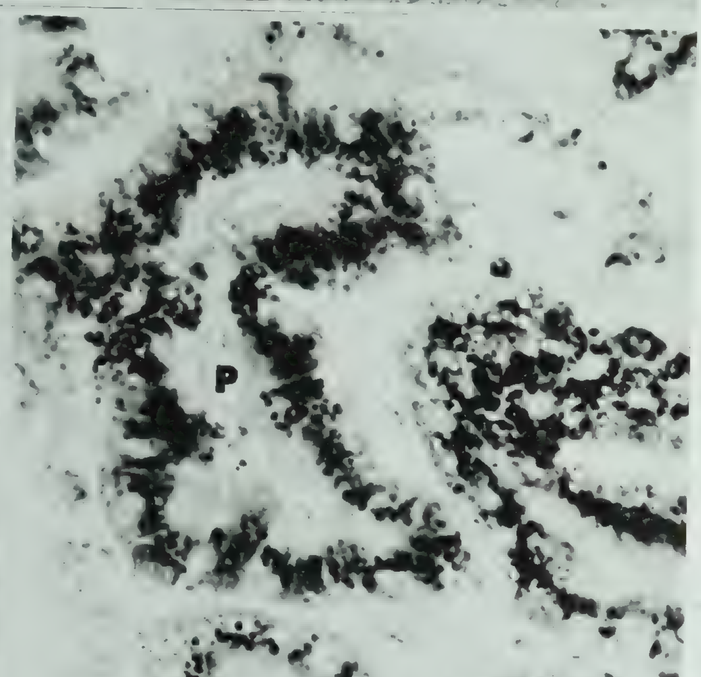

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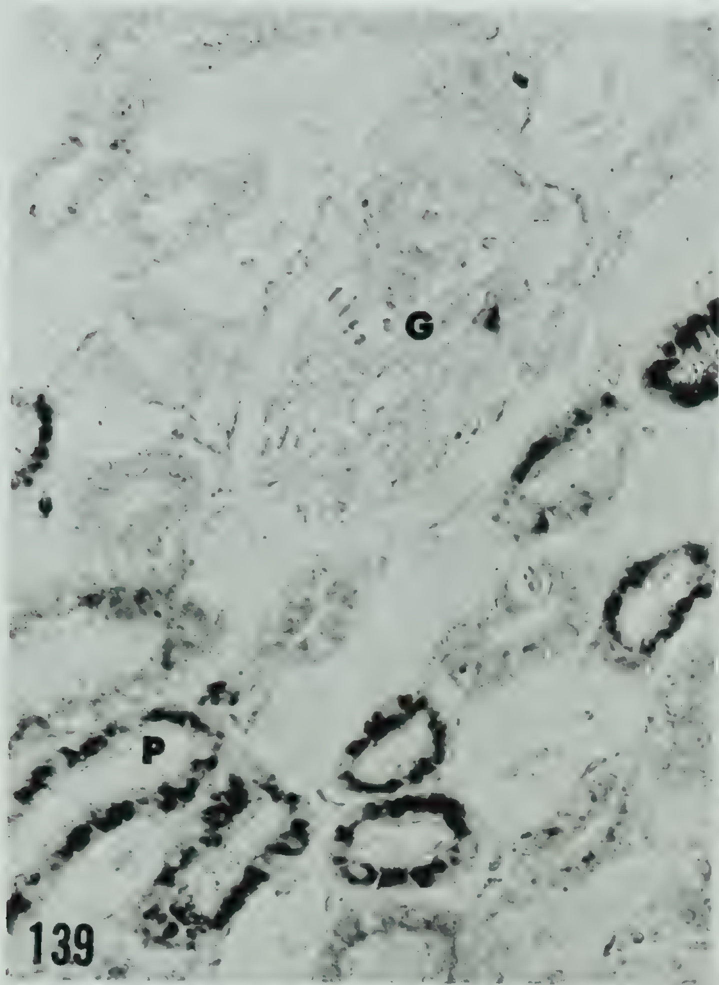
Fig. 139. Grass frog kidney, B-glucuronidase, note the lysosomal enzyme activity in the proximal convoluted tubules (P). 250 X.

Fig. 140. Grass frog kidney, glucosaminidase. 250 X.

Fig. 141. Grass frog kidney, Naphthol AS acetate esterase. 250 X.

Fig. 142. Tree frog kidney, B-glucuronidase. 250 X.



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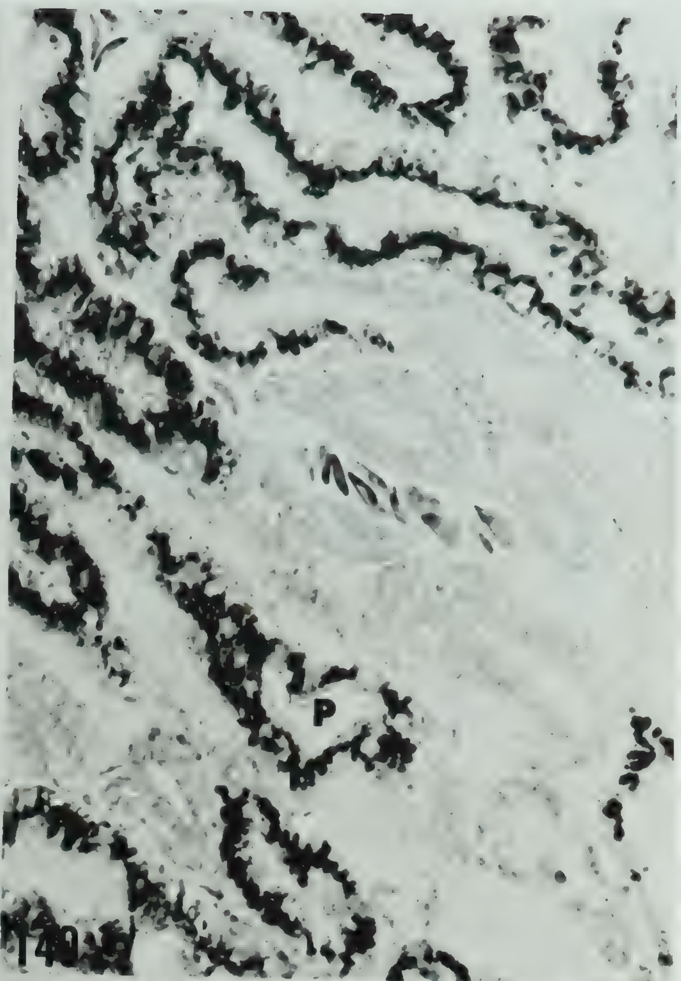

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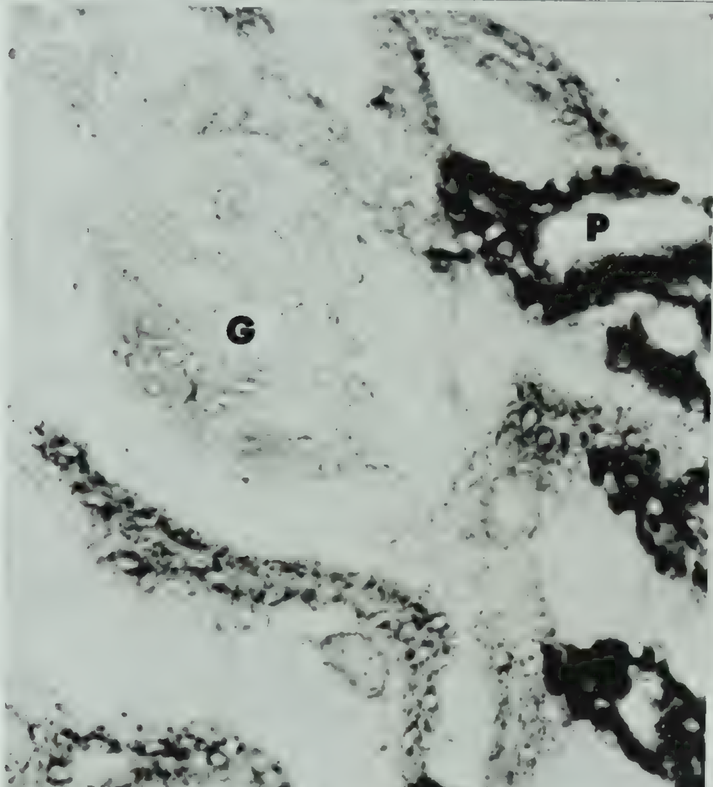

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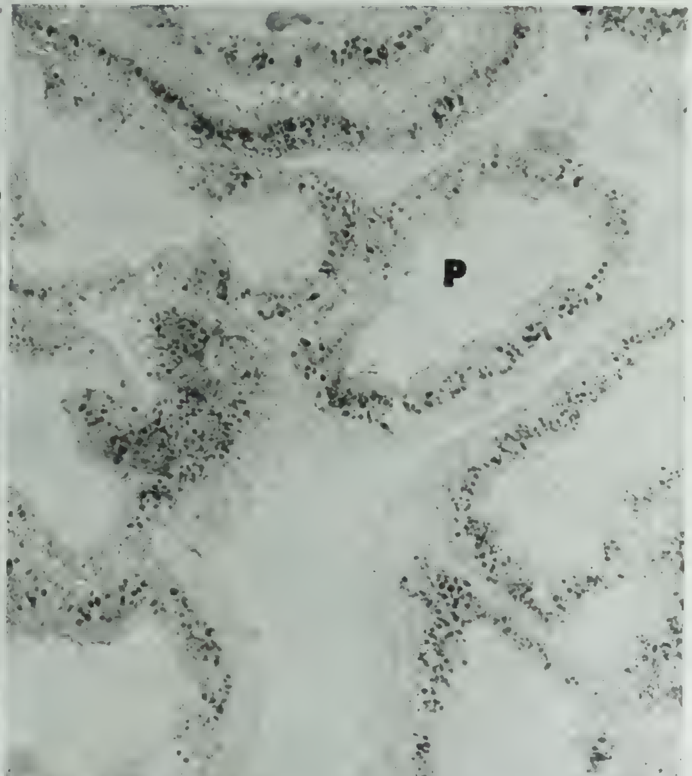

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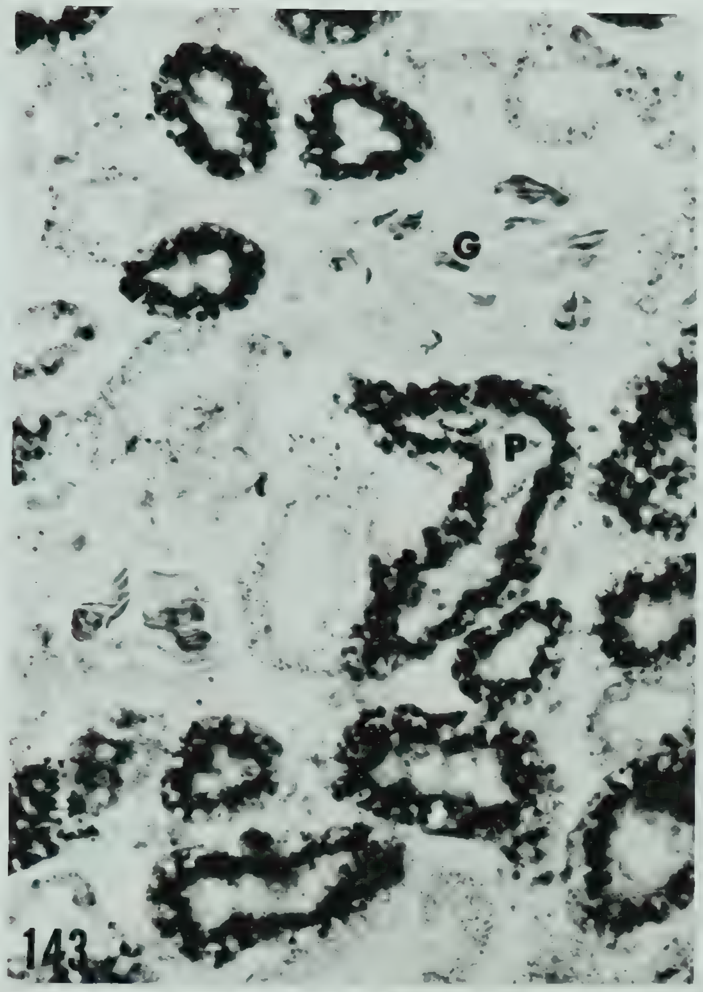
Fig. 143. Tree frog kidney, Naphthol AS acetate esterase. 250 X.

Fig. 144. Grass frog liver, B-glycerophosphate phosphatase, note the pigment bodies (Pb). 250 X.

Fig. 145. Grass frog liver, B-glucuronidase. 250 X.

Fig. 146. Tree frog liver, B-glucuronidase. 250 X.



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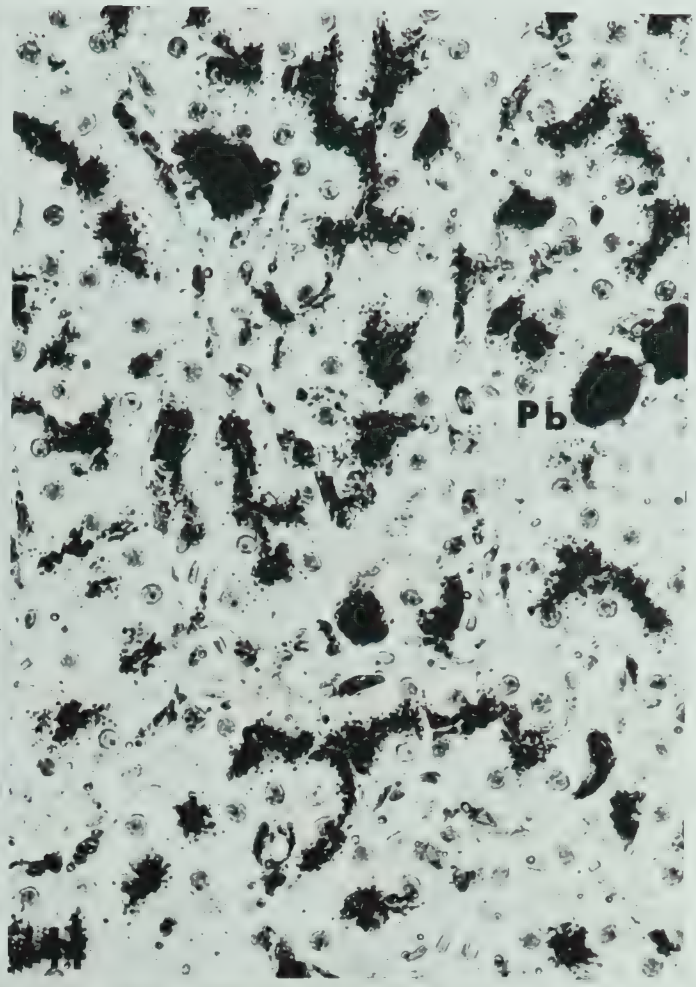

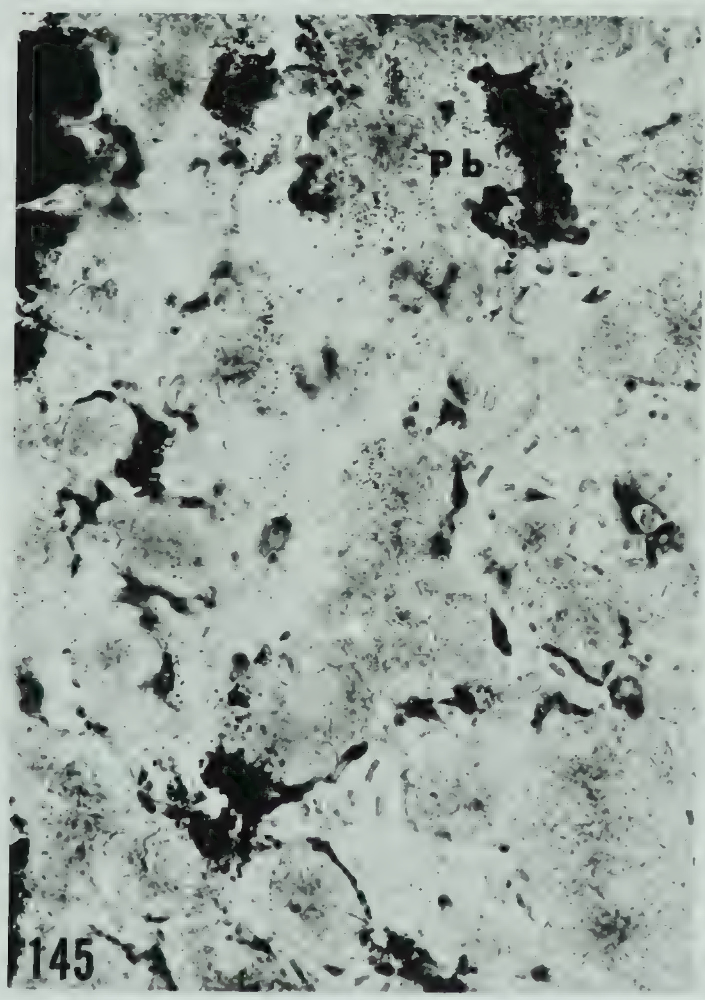

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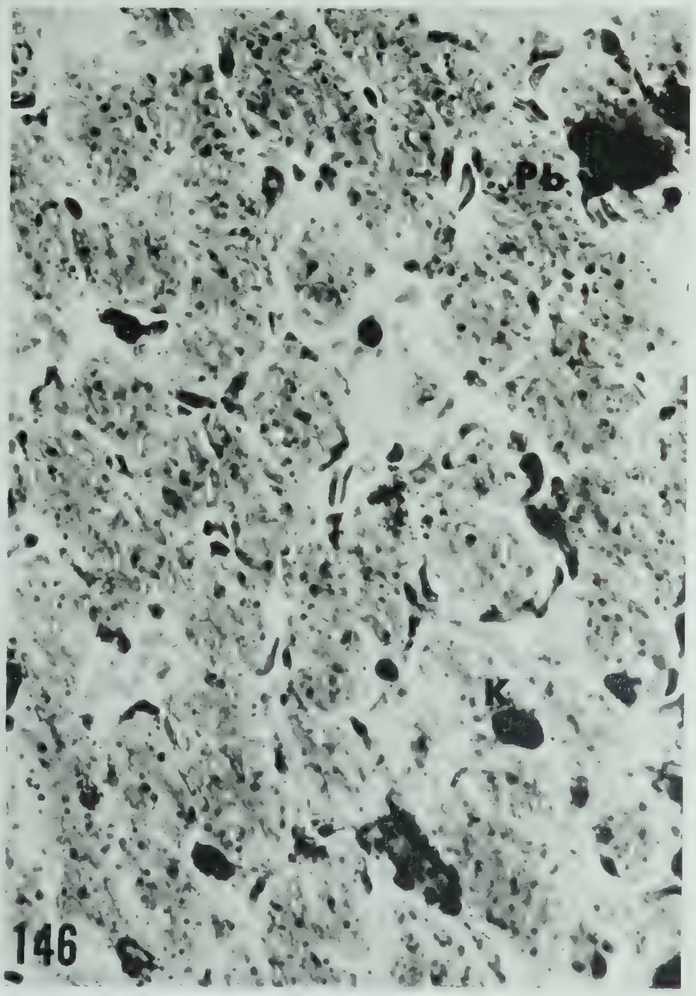

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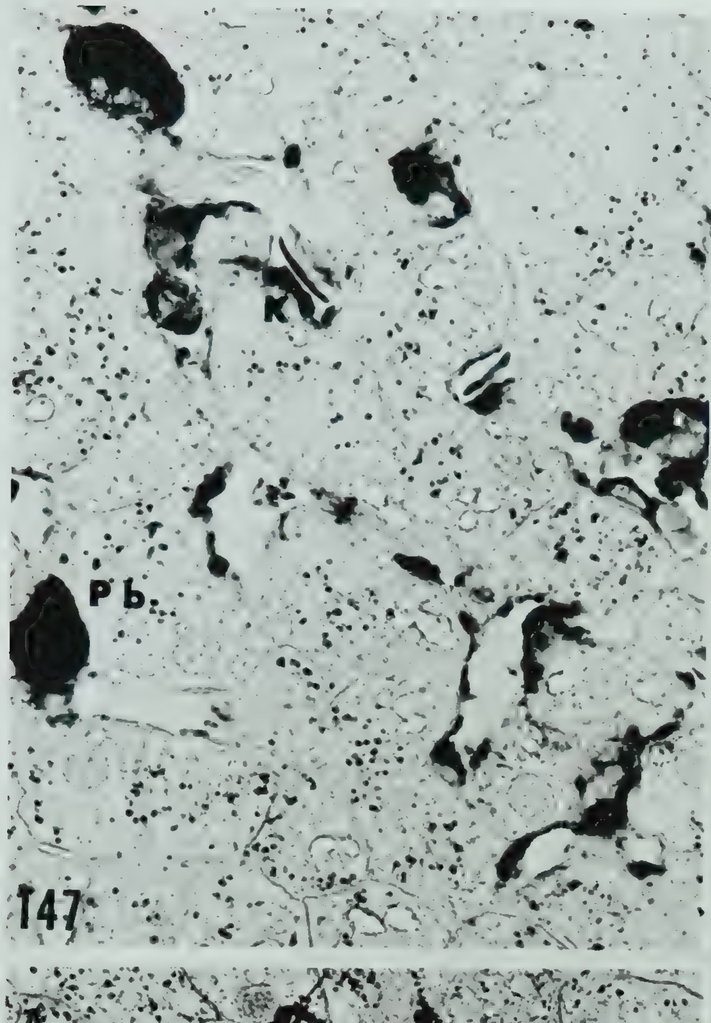
Fig. 147. Salamander liver, glucosaminidase. 250 X.

Fig. 148. Salamander liver, B-glucuronidase. 250 X.

Fig. 149. Salamander liver, B-glycerophosphate phosphatase. 250 X.

Fig. 150. Salamander liver, Naphthol AS-TR phosphate phosphatase. 250 X.



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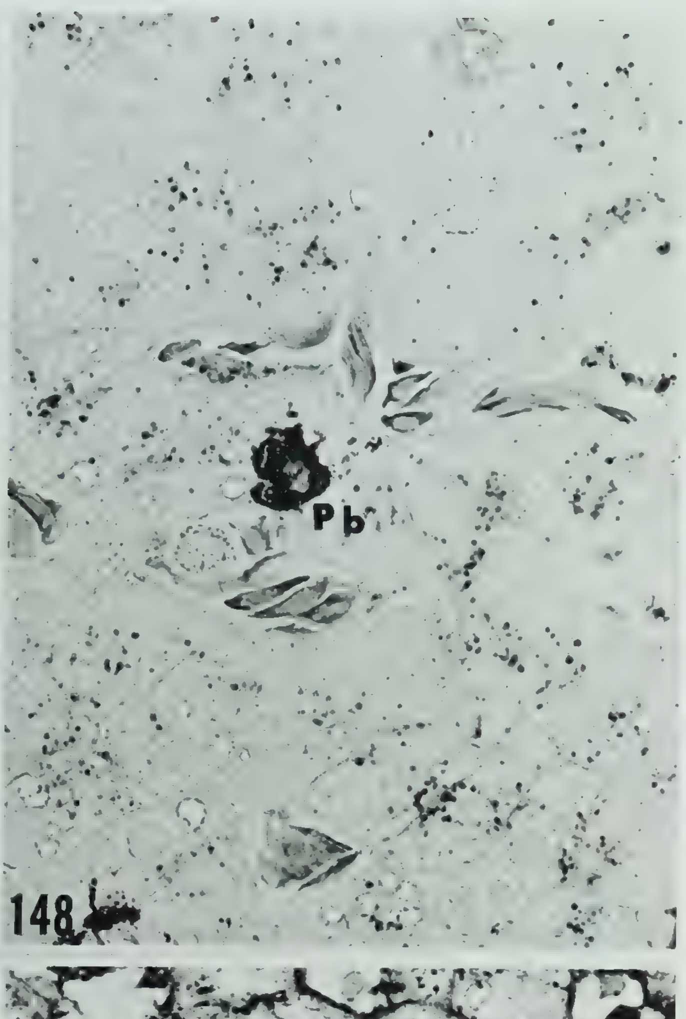

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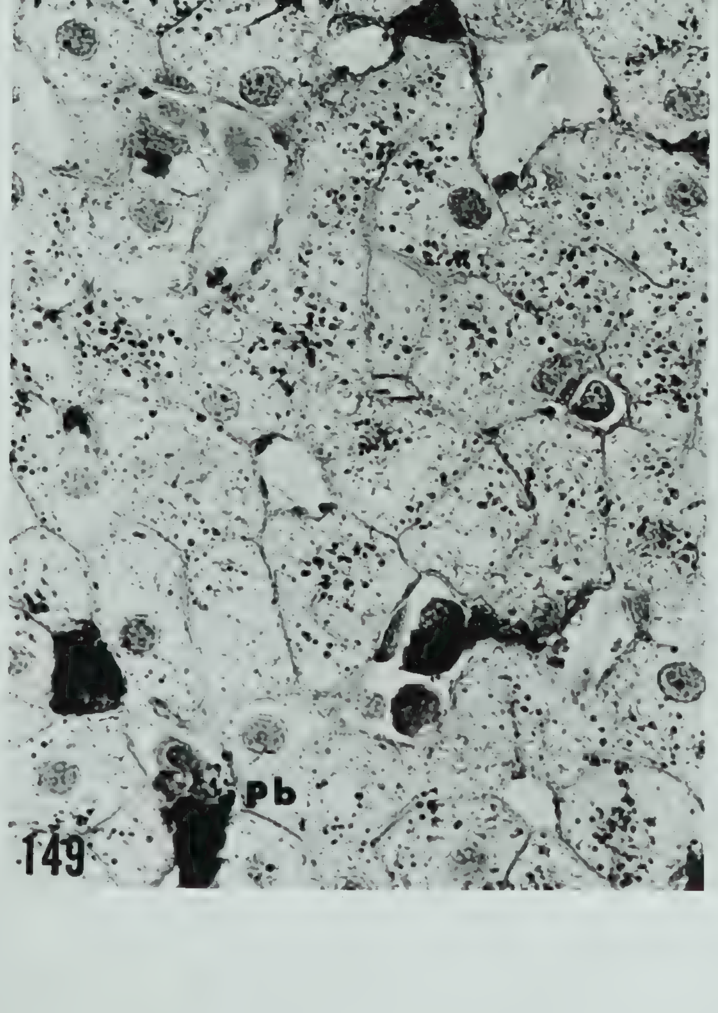

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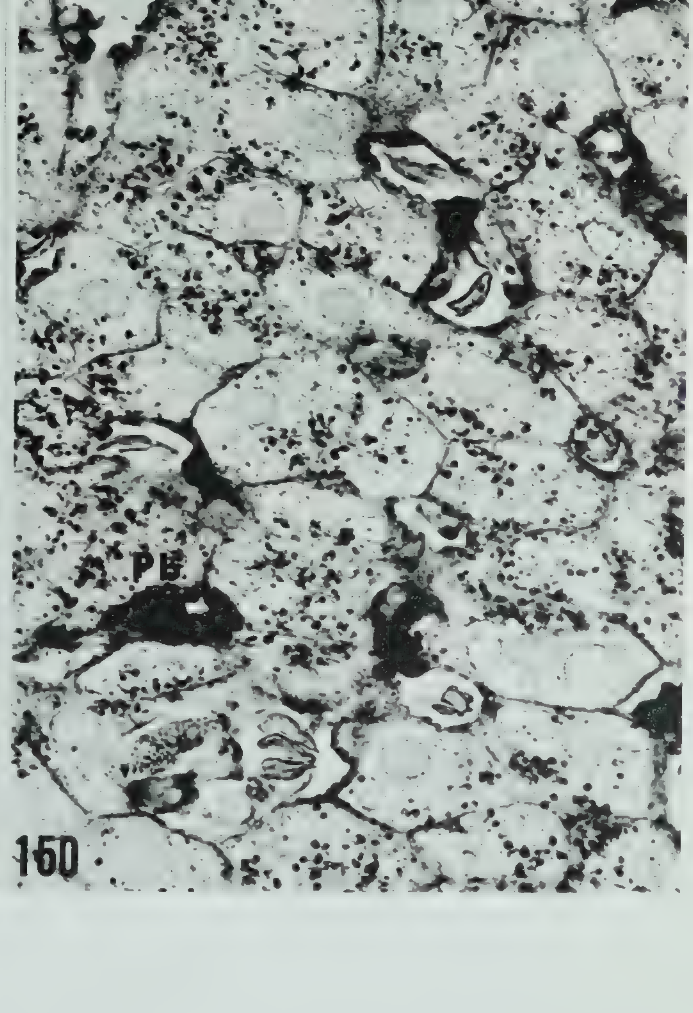

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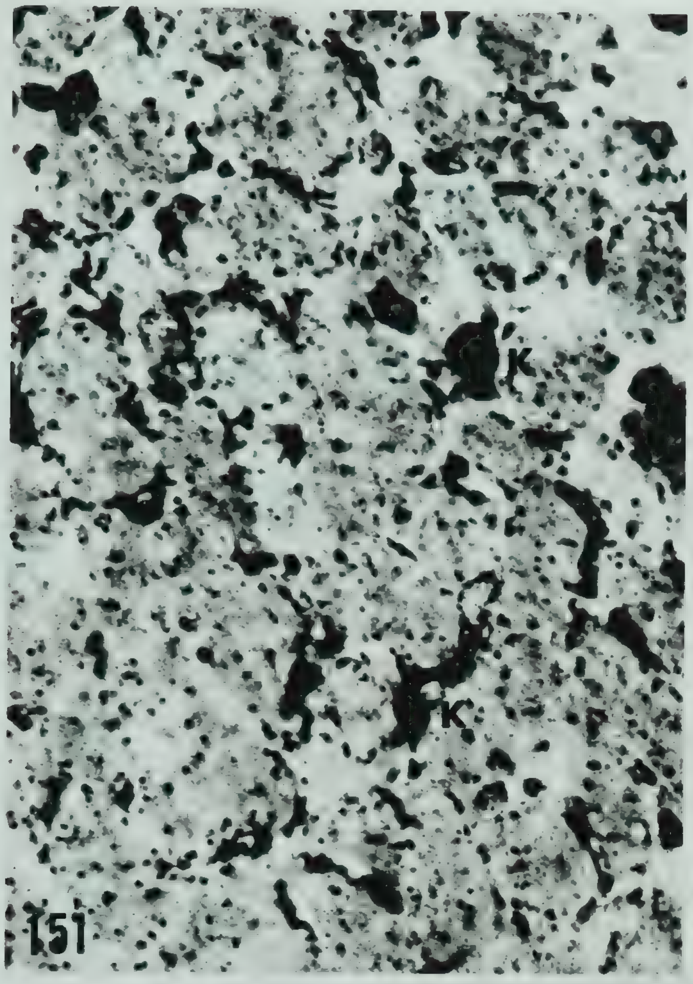
Fig. 151. Tree frog liver, Naphthol AS acetate esterase, note the intense enzyme activity in the Kupffer cells (K). 250 X.

Fig. 152. Grass frog duodenum, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 153. Grass frog duodenum, glucosaminidase. 250 X.

Fig. 154. Tree frog duodenum, B-glucuronidase, note the enzyme activity confined to the lysosomes of the macrophages in the lamina propria. 250 X.



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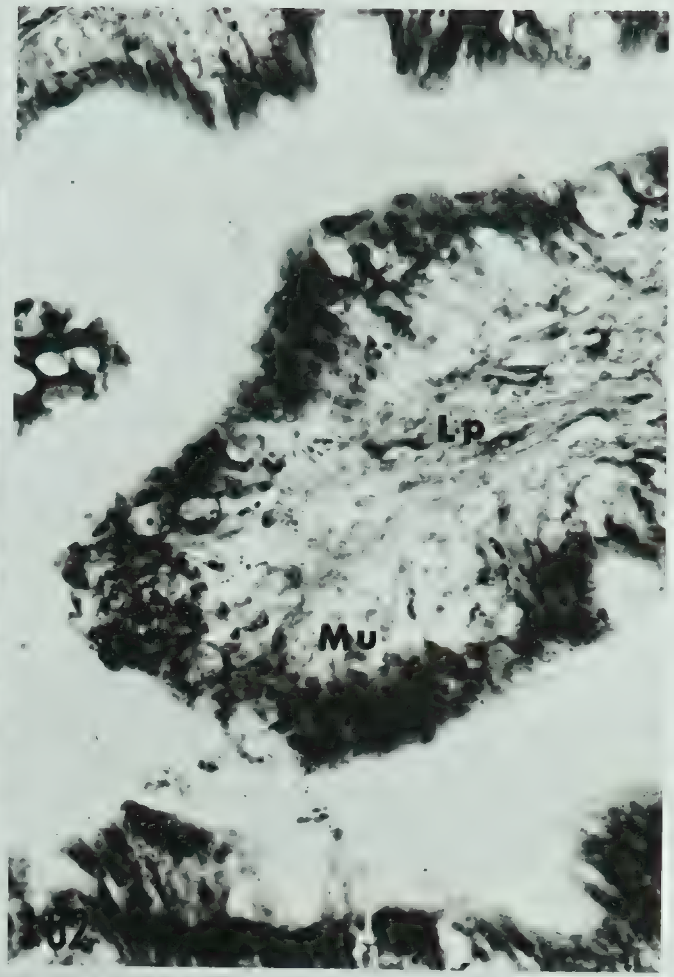

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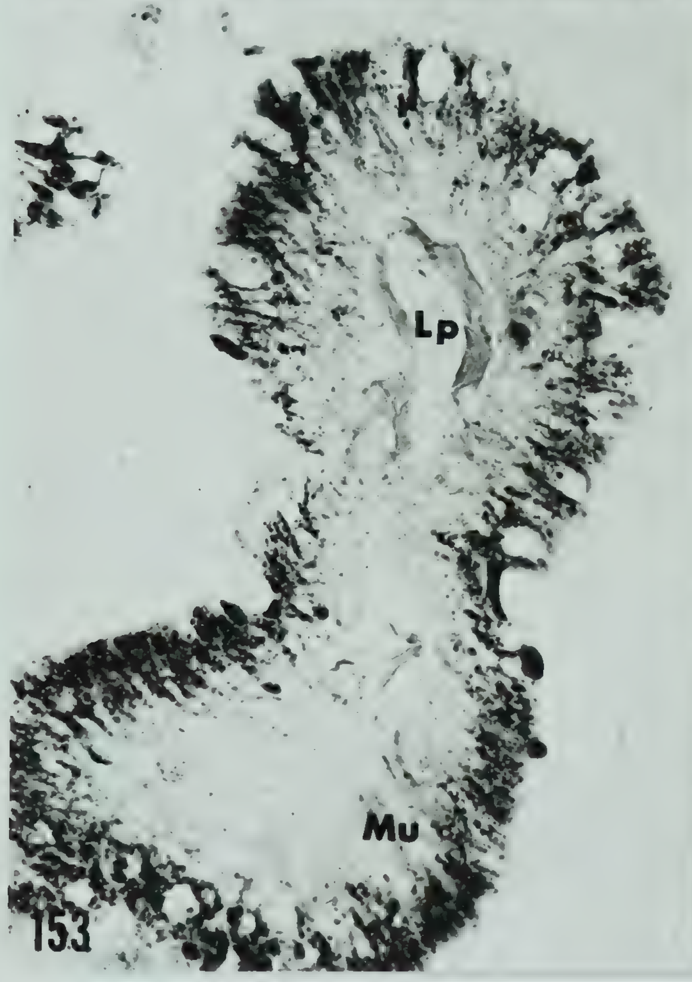

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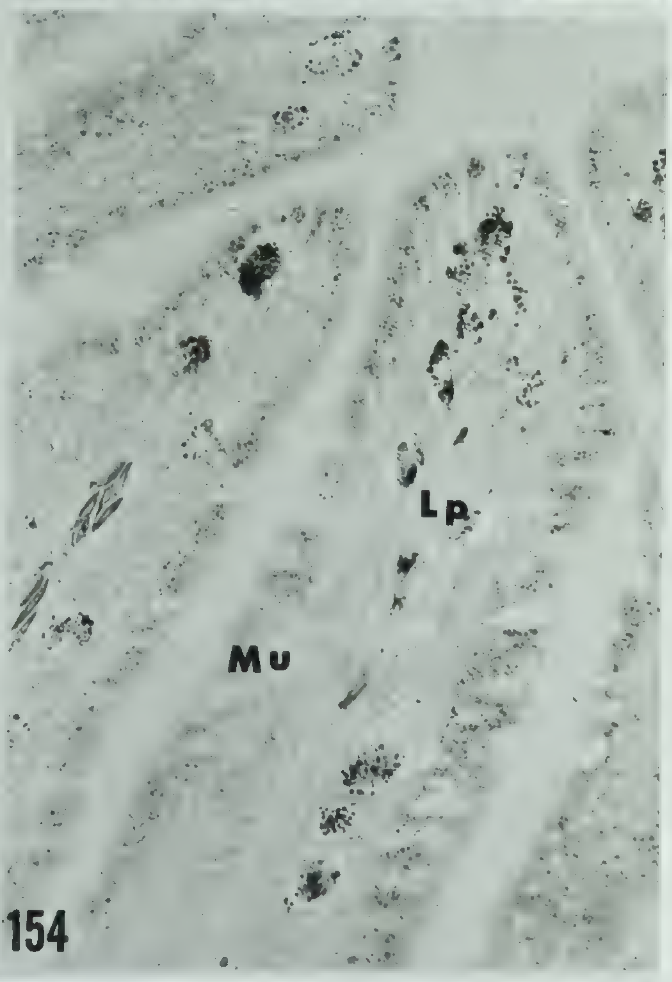

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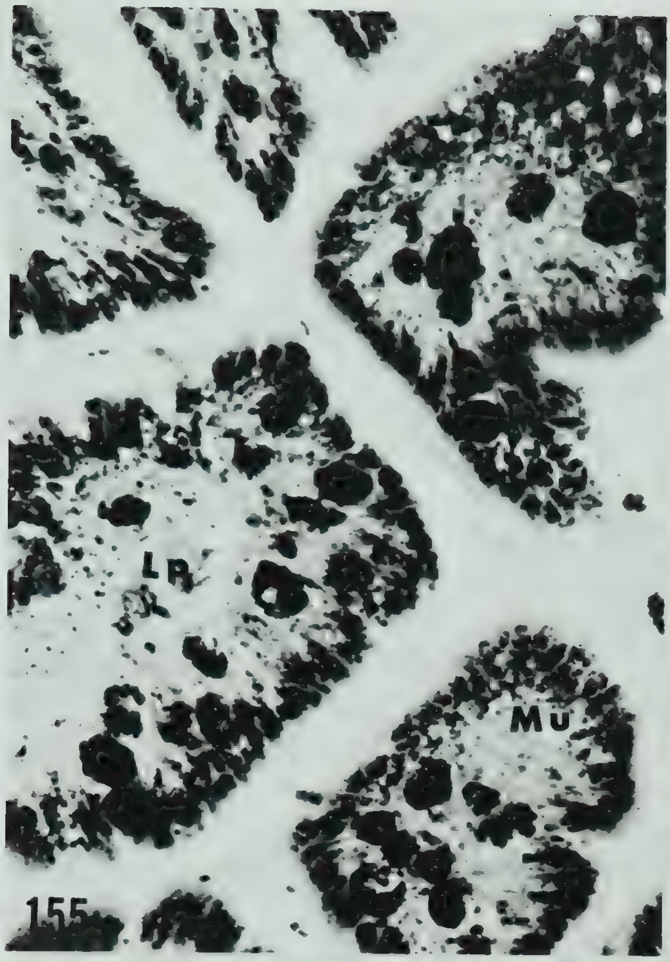
Fig. 155. Tree frog duodenum, Naphthol AS- TR phosphate phosphatase. 250 X.

Fig. 156. Salamander duodenum, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 157. Tree frog pancreas, Naphthol AS acetate esterase. 250 X.



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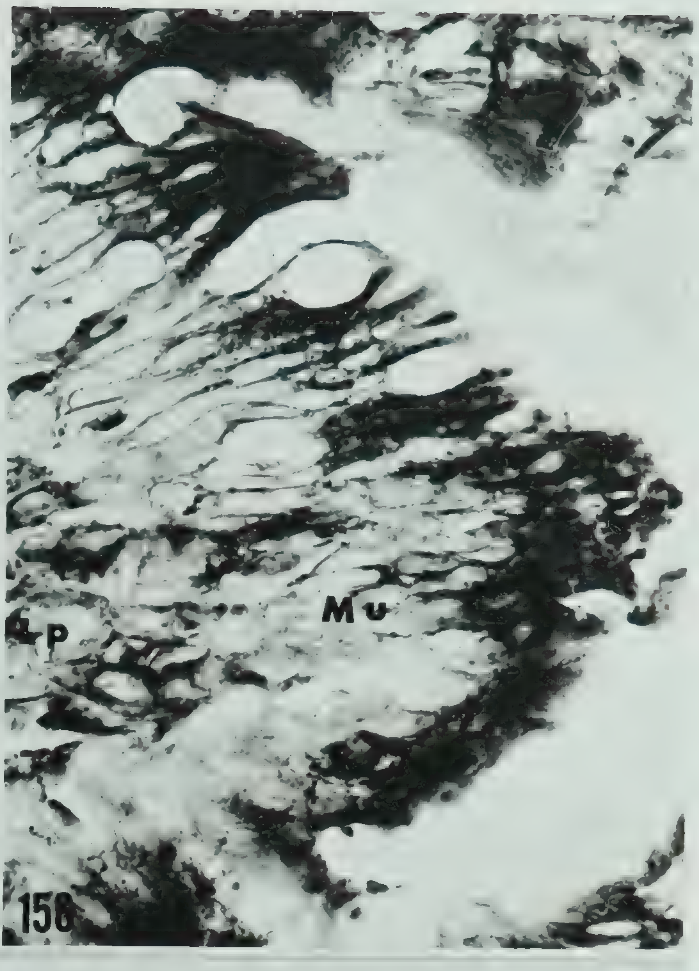

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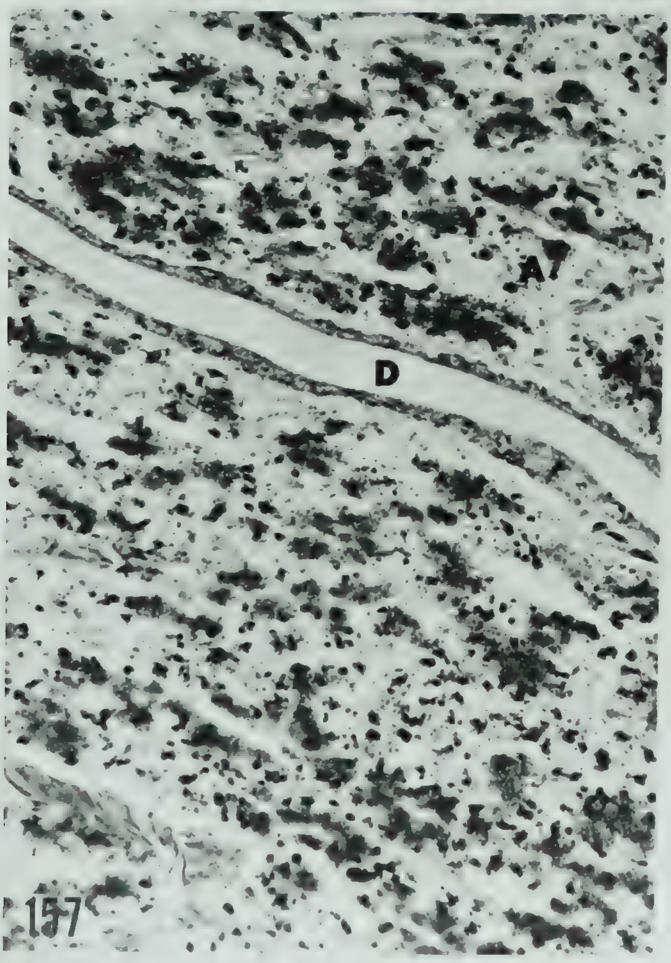

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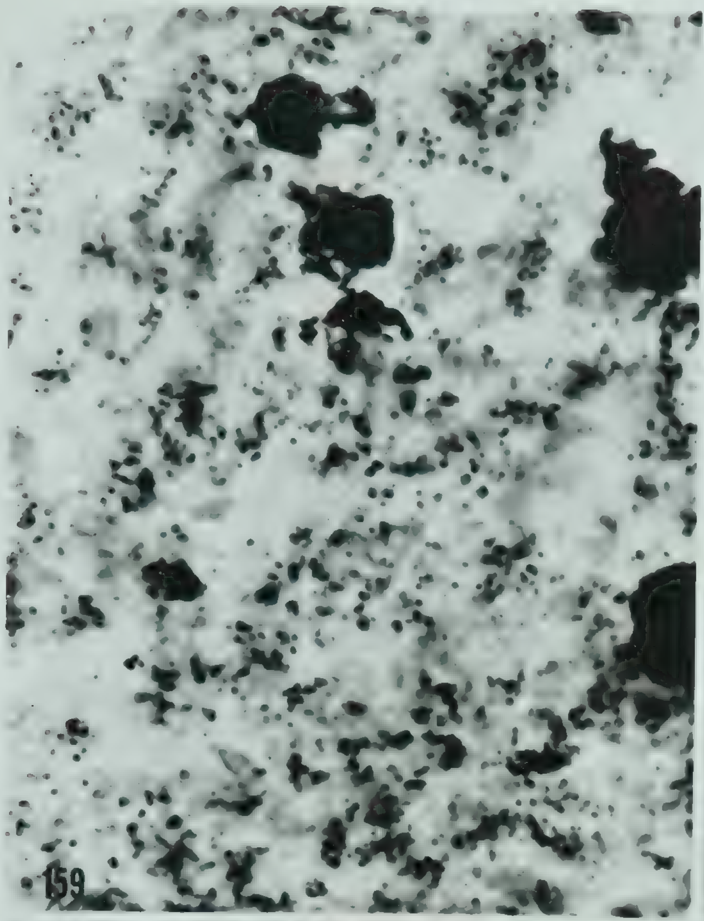
Fig. 159. Newt liver, B-glucuronidase. 250 X.

Fig. 160. Salamander spleen, B-glucuronidase. 250 X.

Fig. 161. Salamander spleen, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 162. Newt spleen, Naphthol AS-TR phosphate phosphatase. 250 X.



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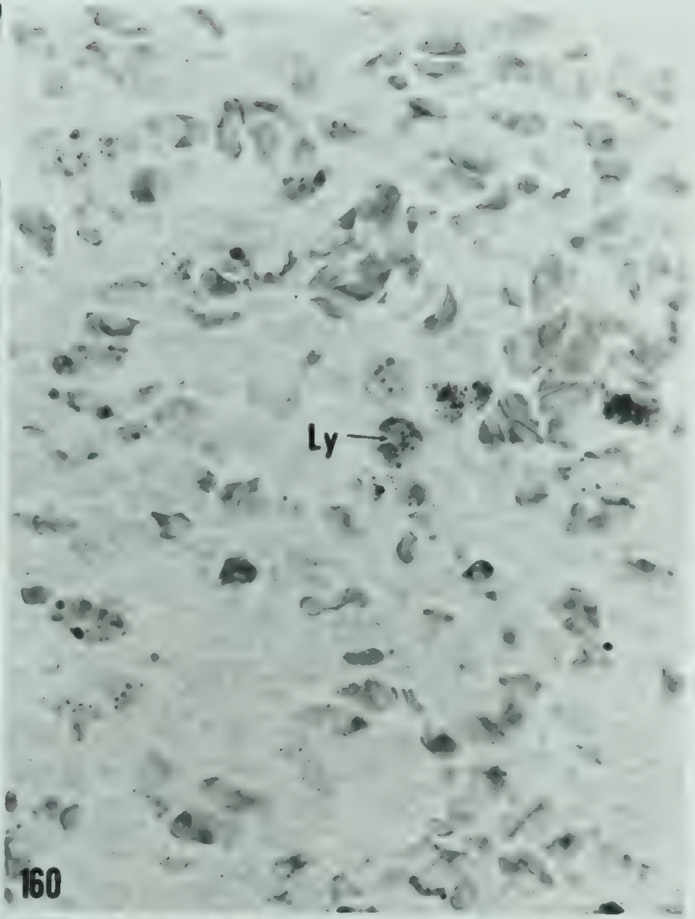

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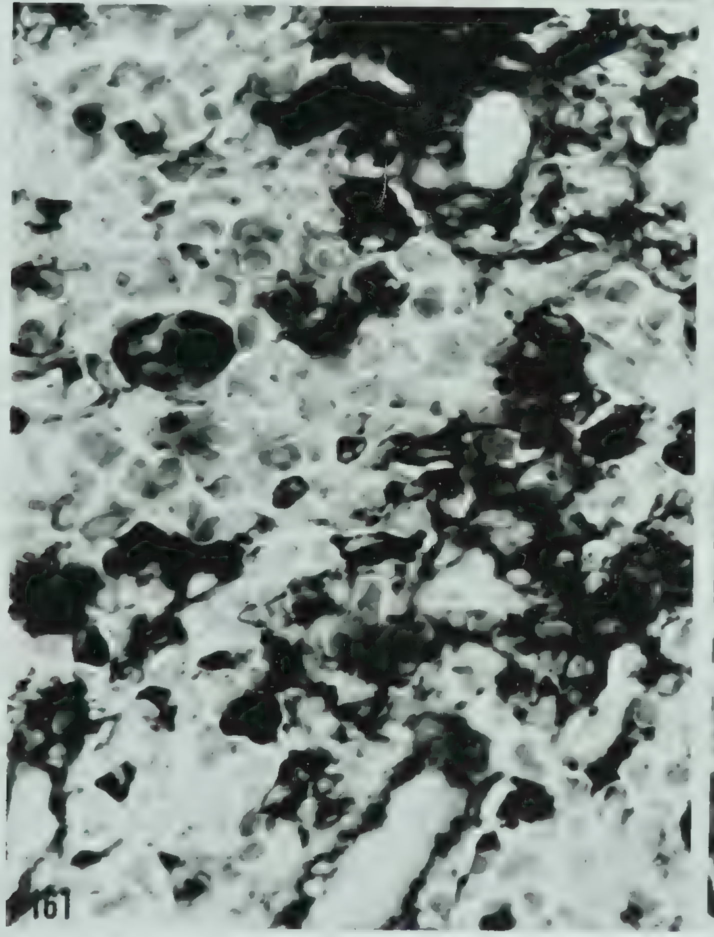

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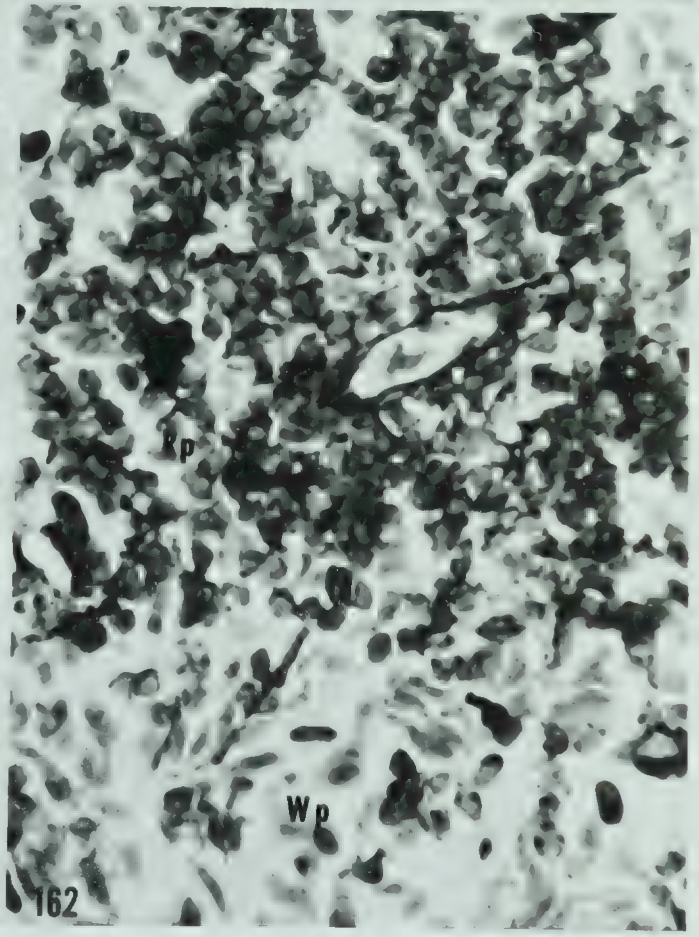

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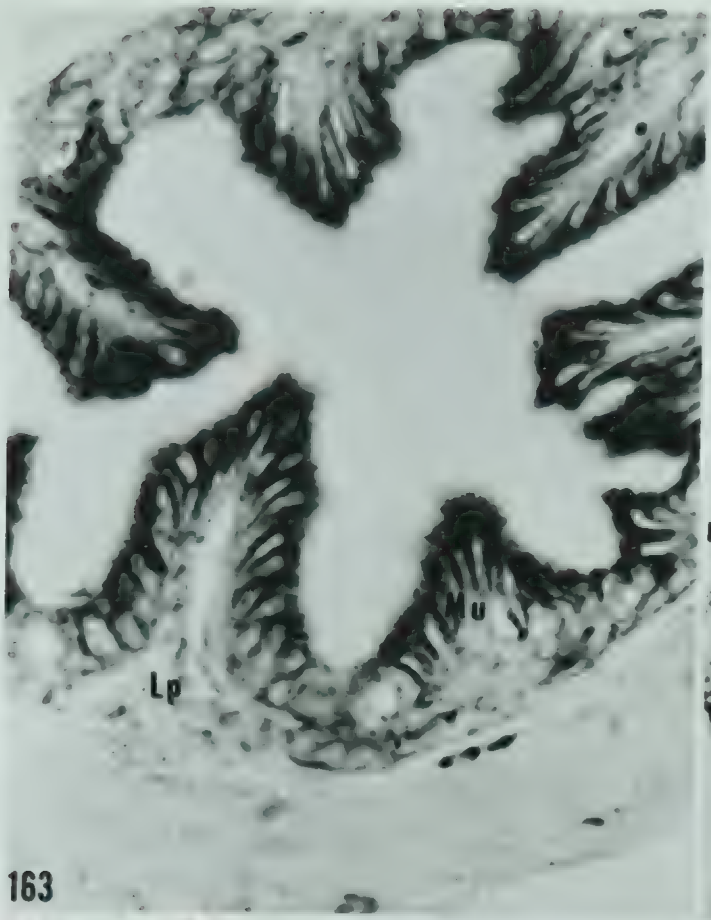
Fig. 163. Newt duodenum, glucosaminidase. 250 X.

Fig. 164. Salamander duodenum, glucosaminidase. 250 X.

Fig. 165. Newt brain, glucosaminidase. 250 X.

Fig. 166. Salamander brain, Naphthol AS-TR phosphate phosphatase, note the polarity of the lysosomes in neurons (Ne). 250 X.



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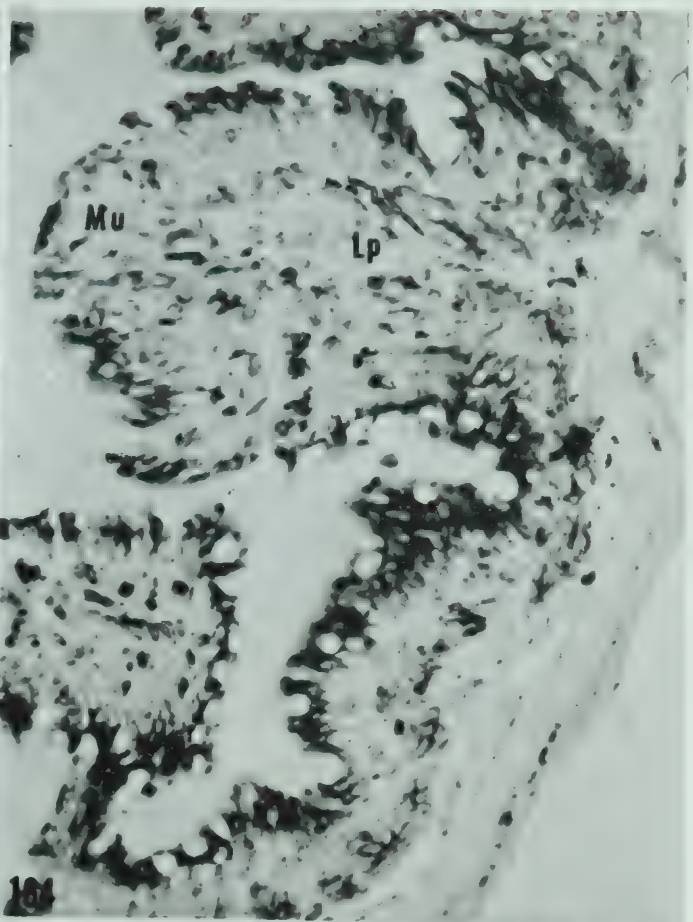

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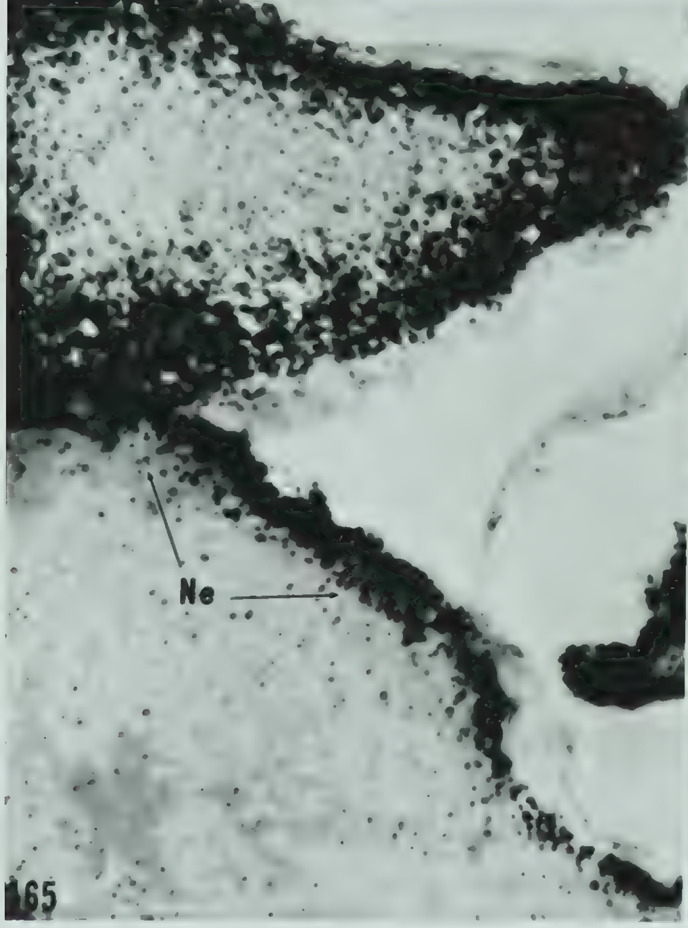

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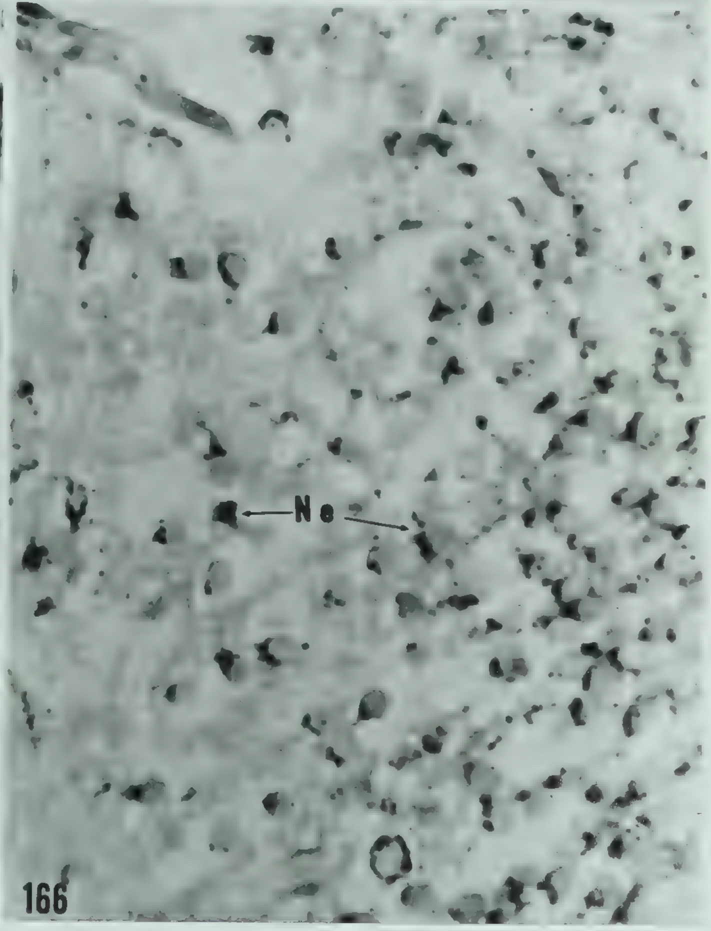

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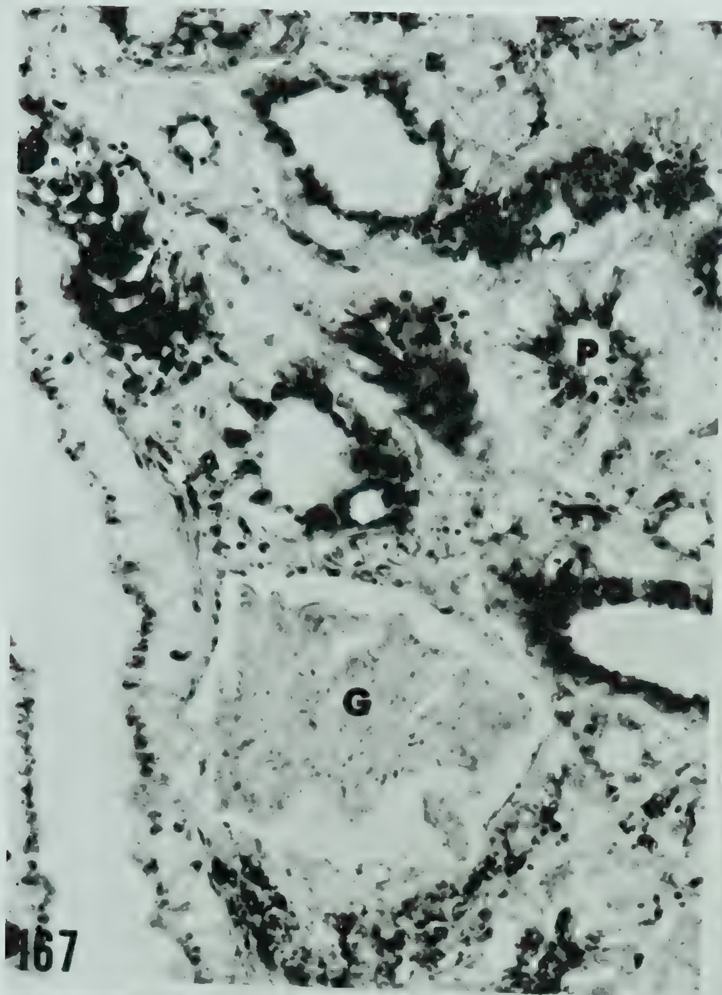
Fig. 167. Esox lucius kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 168. Stizostedion vitreum kidney, Naphthol AS-TR phosphate phosphatase, note extensive lymphoid tissue (Res) between the tubules and the glomerulus (G). 250 X.

Fig. 169. Esox lucius kidney, B-glucuronidase. 250 X.

Fig. 170. Stizostedion vitreum kidney, B-glucuronidase. 250 X.



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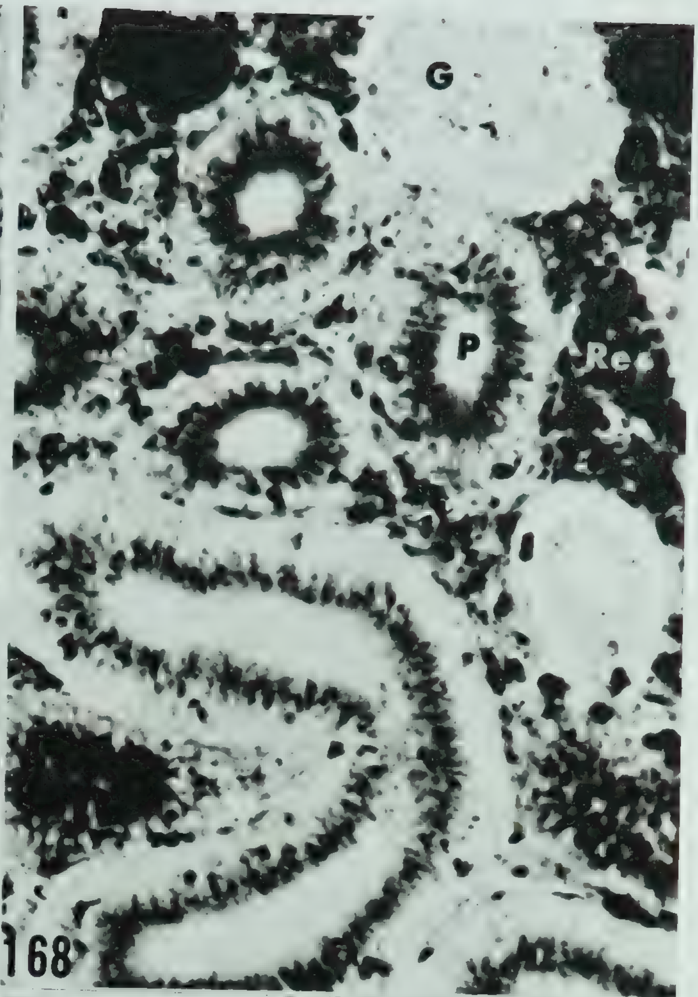

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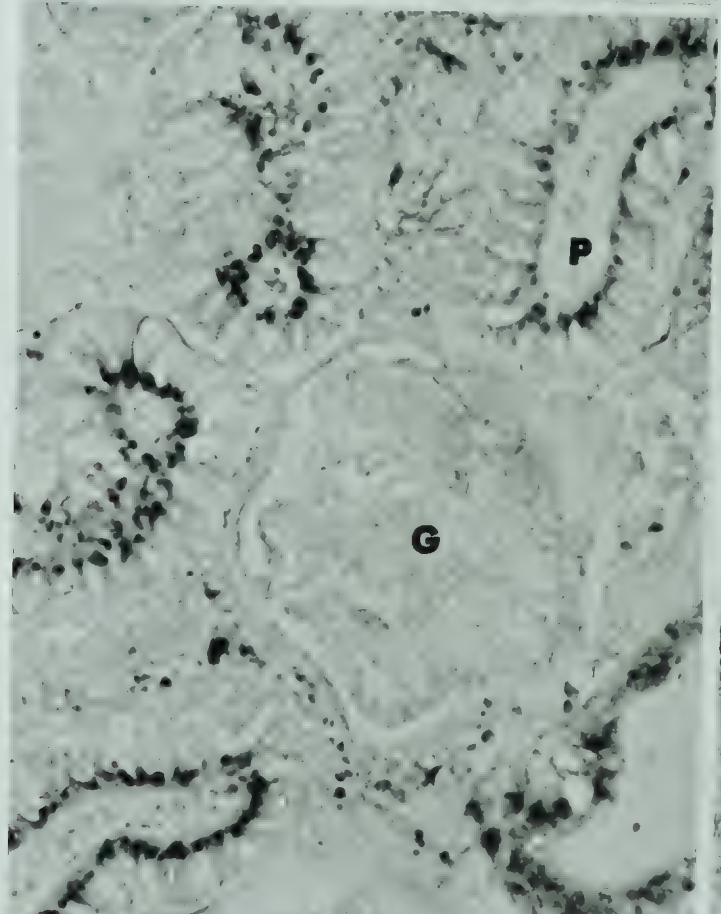

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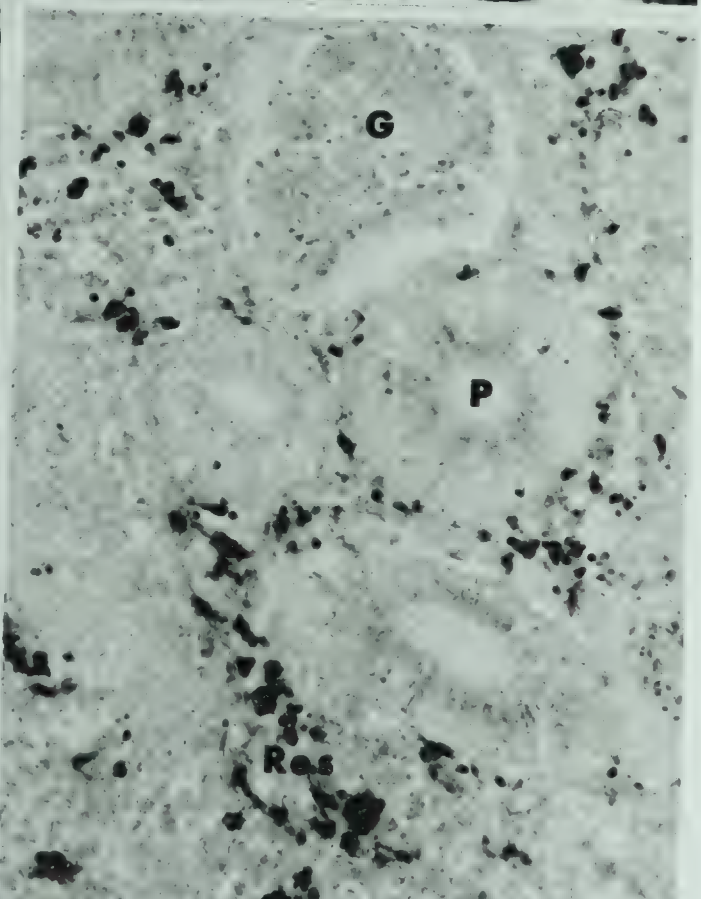

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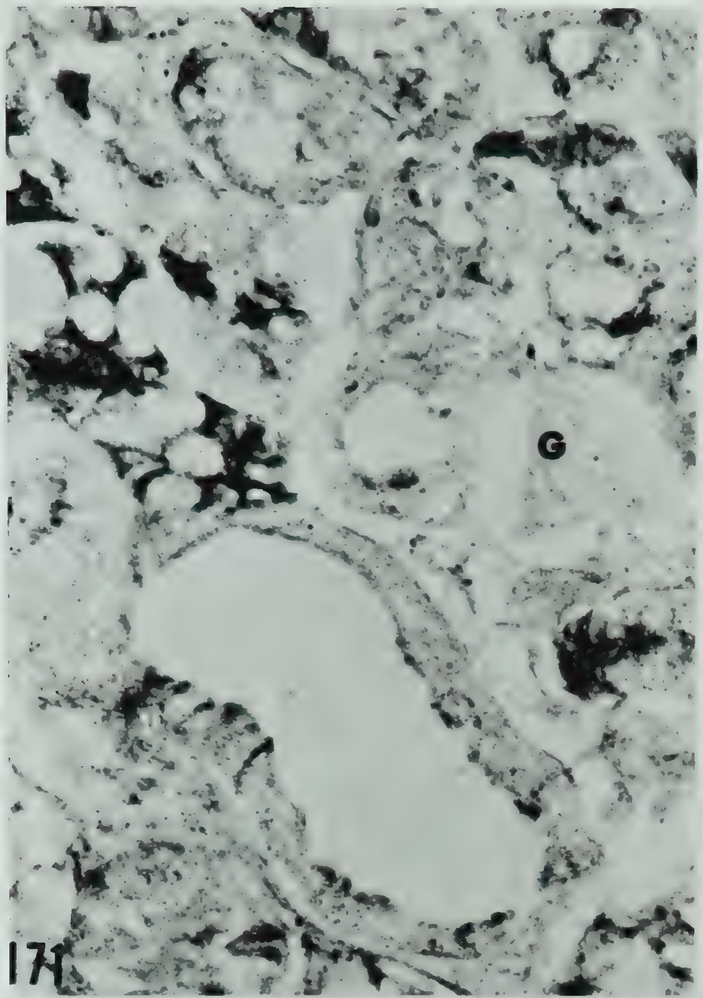
Fig. 171. Esox lucius kidney, Naphthol AS acetate esterase. 250 X.

Fig. 172. Stizostedion vitreum kidney, a-Naphthyl acetate esterase, note the pigment body (Pb) and the extensive lymphoid tissue (Res). 250 X.

Fig. 173. Esox lucius liver, Naphthol AS-TR phosphate phosphatase, note intense enzyme activity in the pericanalicular region of the liver parenchymal cells. 250 X.

Fig. 174. Stizostedion vitreum liver, Naphthol AS-TR phosphate phosphatase. 250 X.



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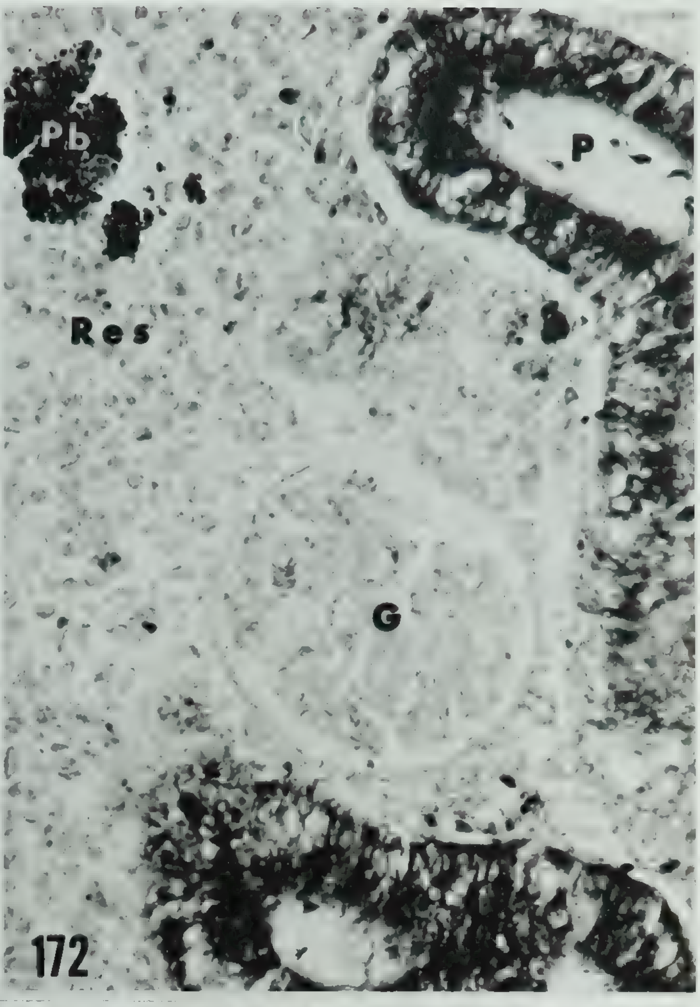

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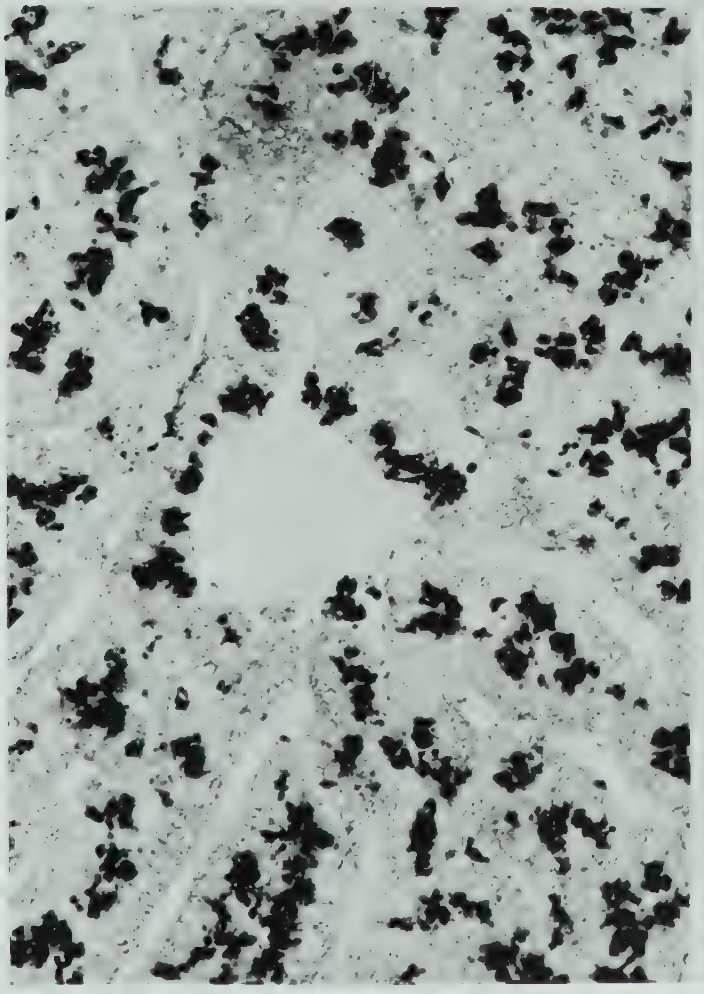

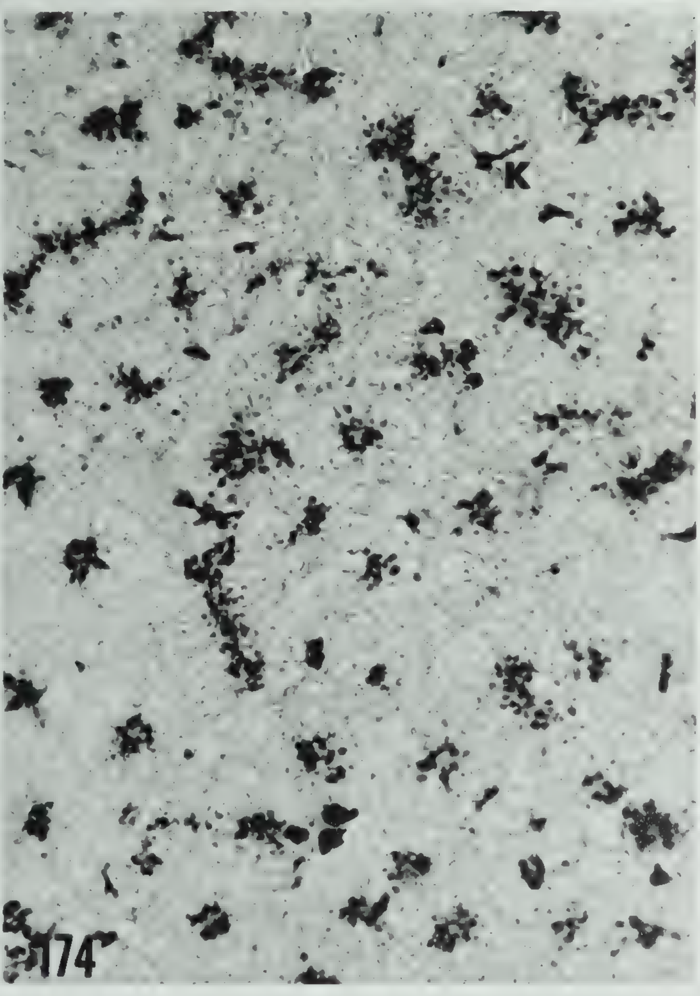

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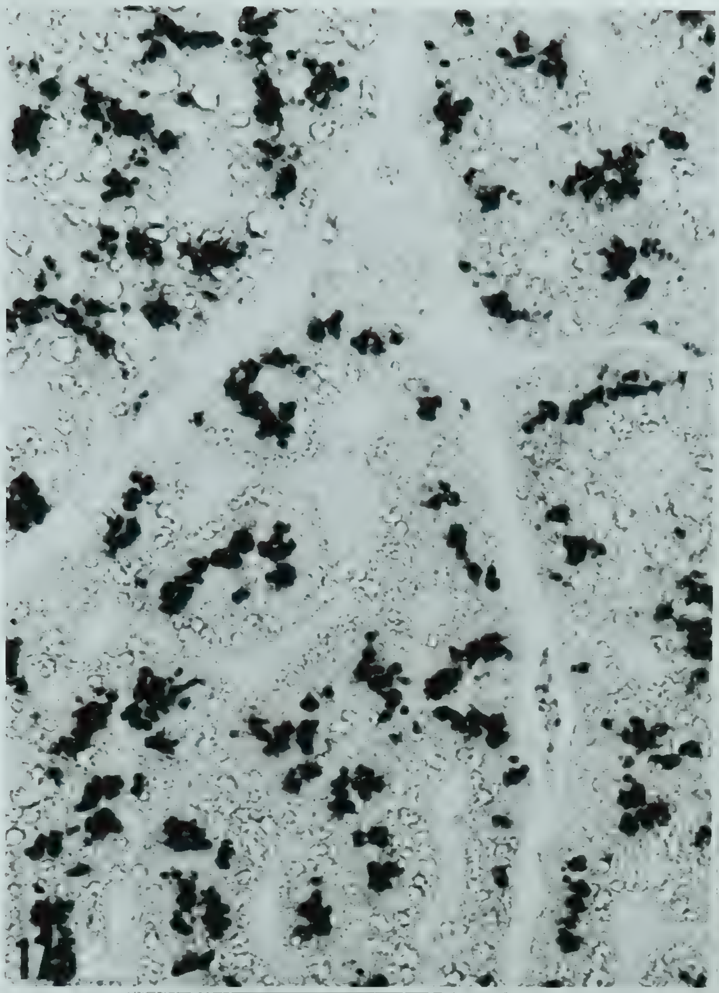
Fig. 175. Esox lucius liver, B-glucuronidase. 250 X.

Fig. 176. Stizostedion vitreum liver, a-Naphthyl acetate esterase. 250 X.

Fig. 177. Esox lucius duodenum, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 178. Esox lucius duodenum, glucosaminidase. 250 X.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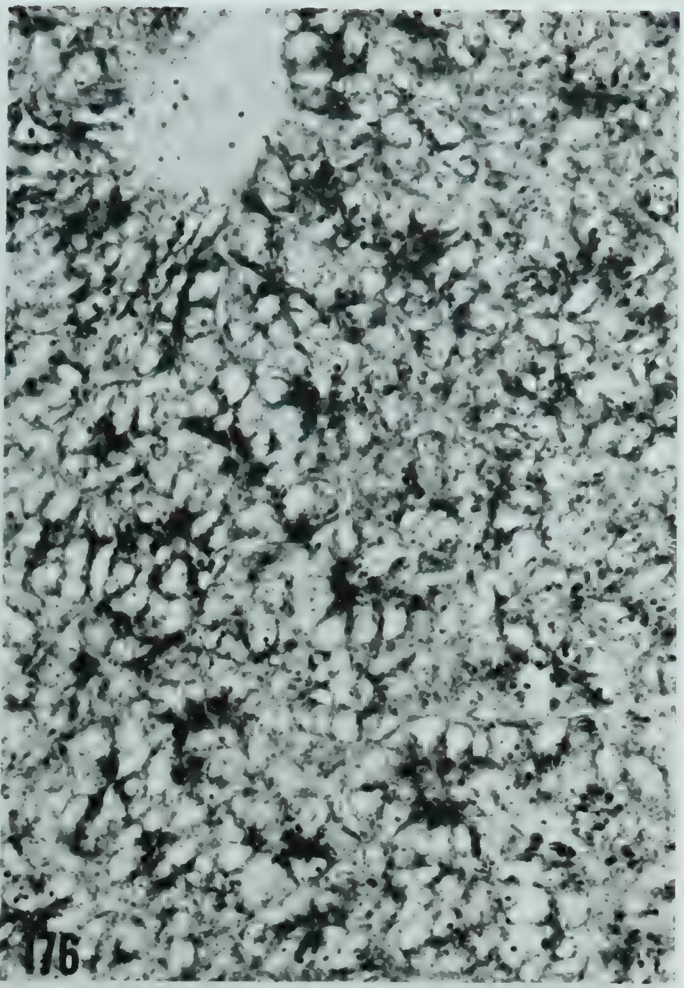
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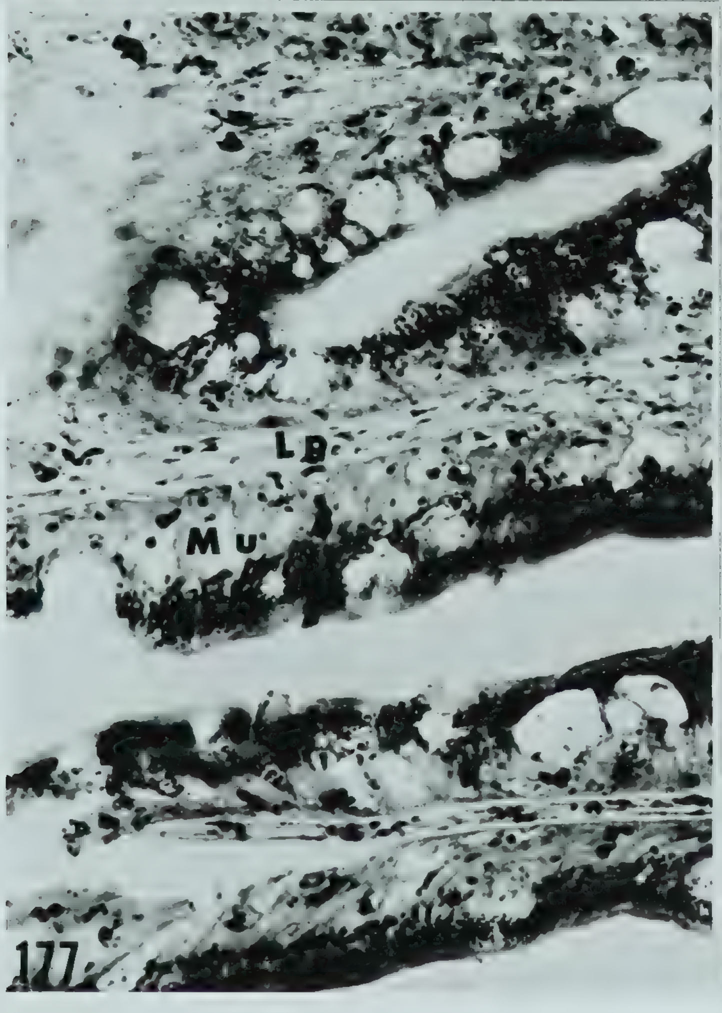
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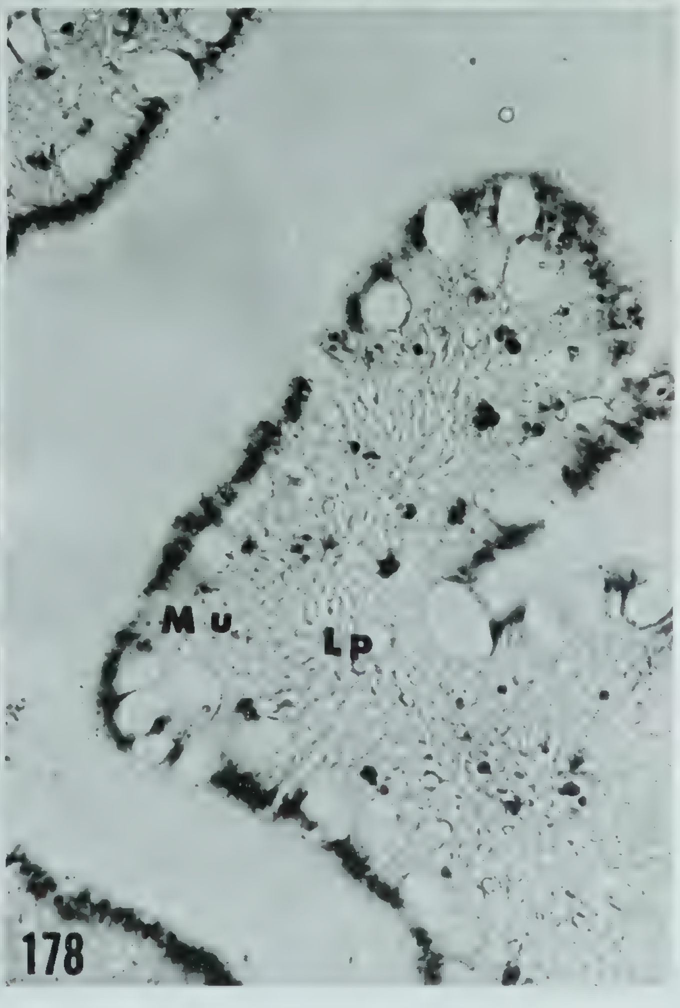
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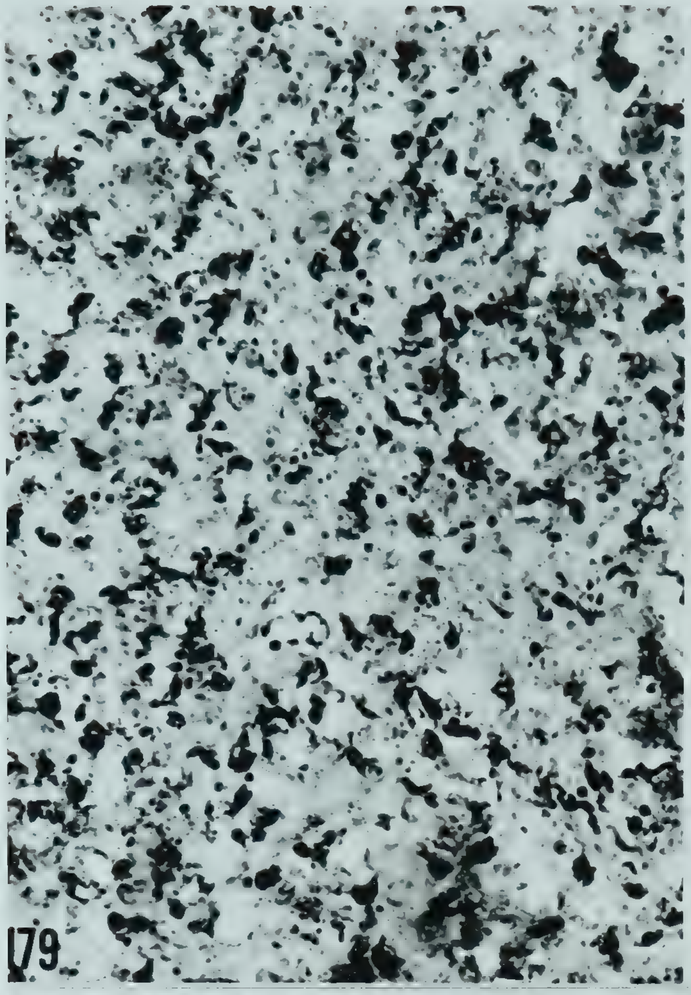
Fig. 179. Esox lucius spleen, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 180. Esox lucius spleen, glucosaminidase. 250 X.

Fig. 181. Stizostedion vitreum spleen, Naphthol AS-TR phosphate phosphatase, note the pigment body (Pb). 250 X.

Fig. 182. Stizostedion vitreum spleen, glucosaminidase. 250 X.



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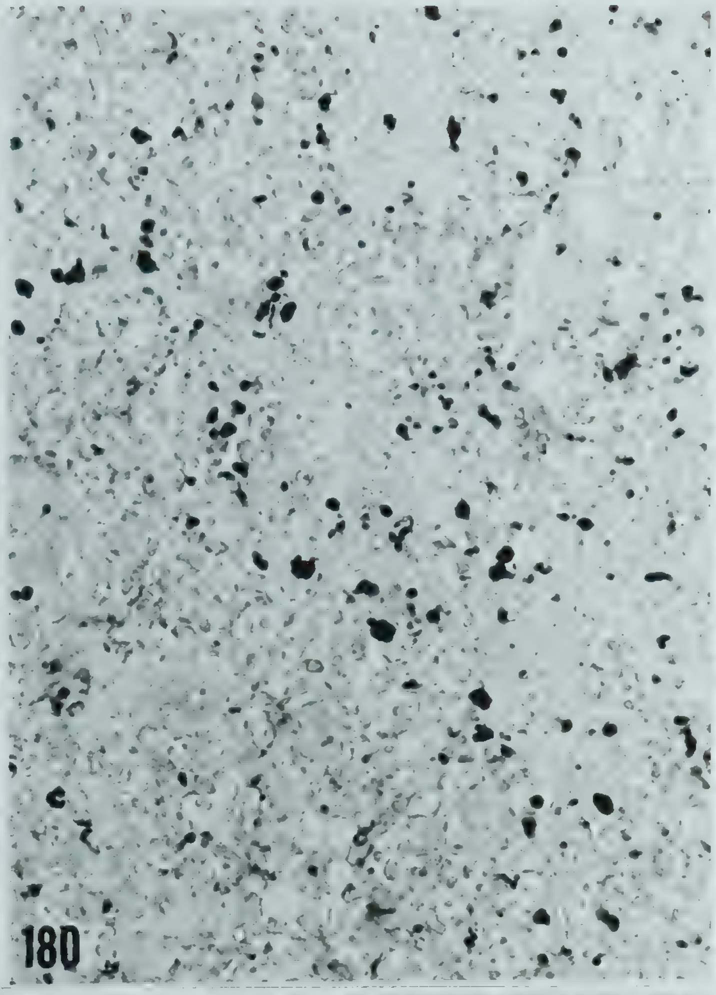

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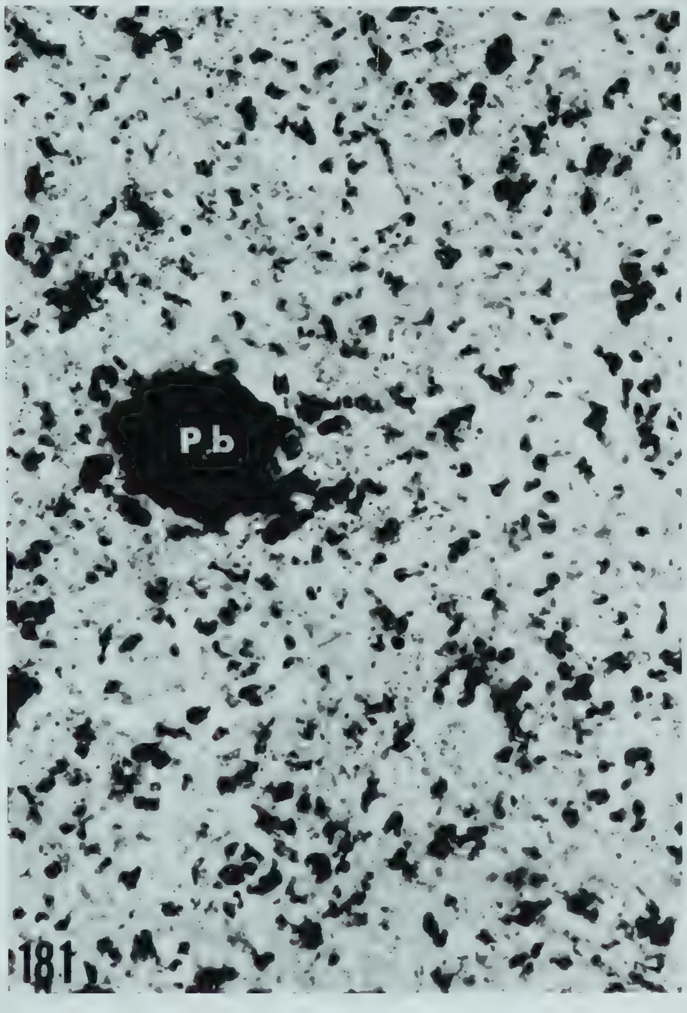

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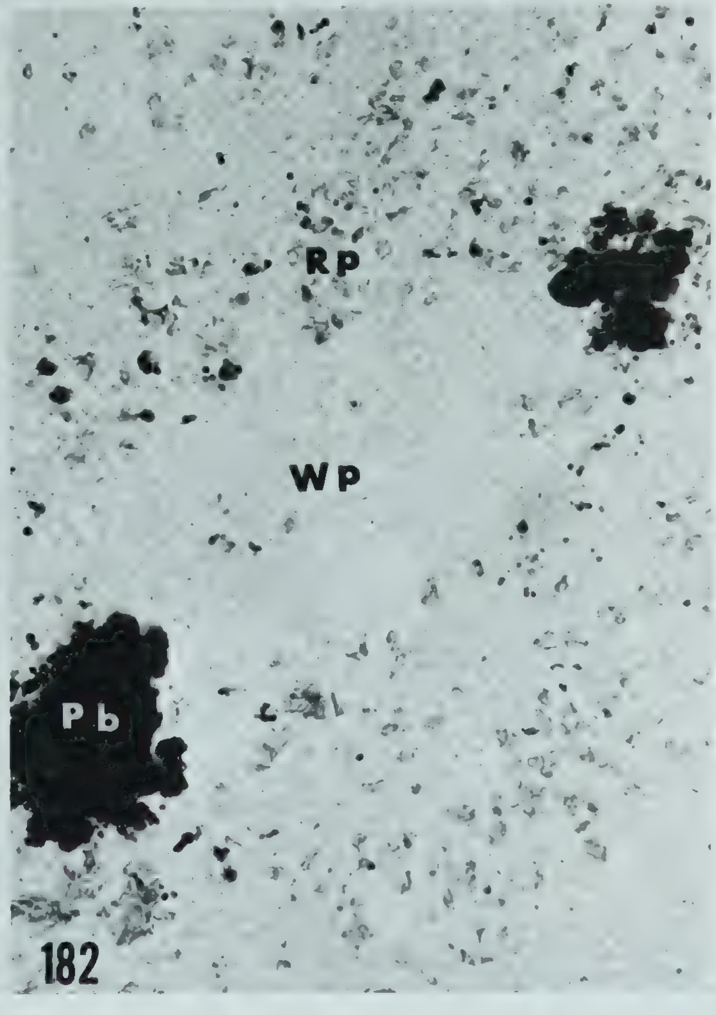

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Photomicrographs of Histochemical Localization of Lysosomal Acid Hydrolases in Rodent Tissues Altered by Experimental Means.

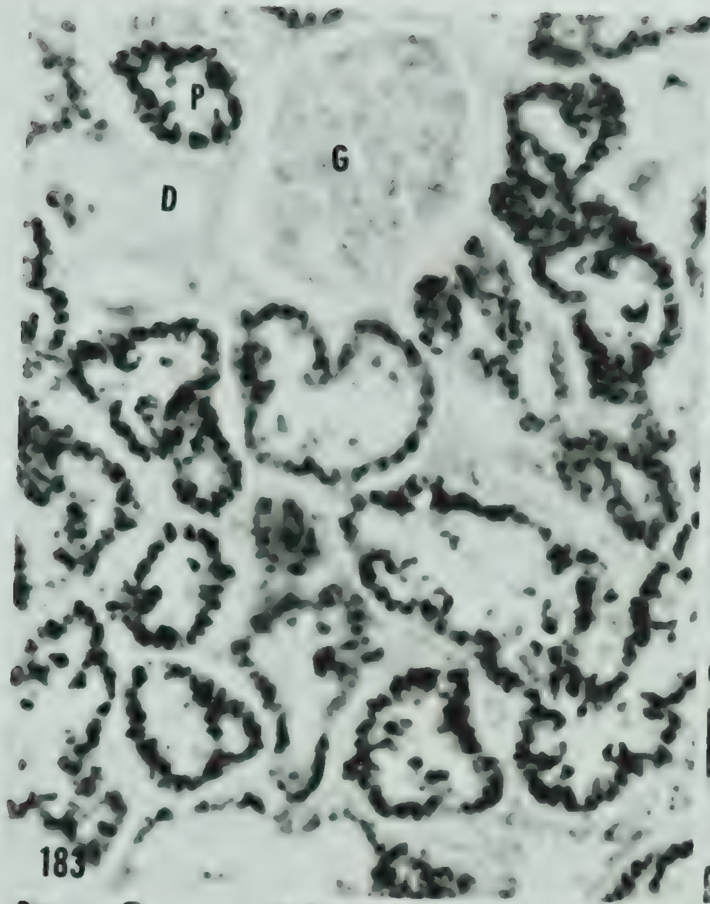
Fig. 183. to Fig. 252. inclusive.

Fig. 183. Normal rat kidney, control for phenacetin experiment, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 184. Normal rat kidney, control for phenacetin experiment, B-glycerophosphate phosphatase. 250 X.

Fig. 185. Phenacetin experiment, (1.8 % dietary administration of phenacetin for 9 weeks), rat kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 186. Phenacetin experiment, (1.8 % dietary administration of phenacetin for 9 weeks), rat kidney, B-glycerophosphate phosphatase. 250 X.



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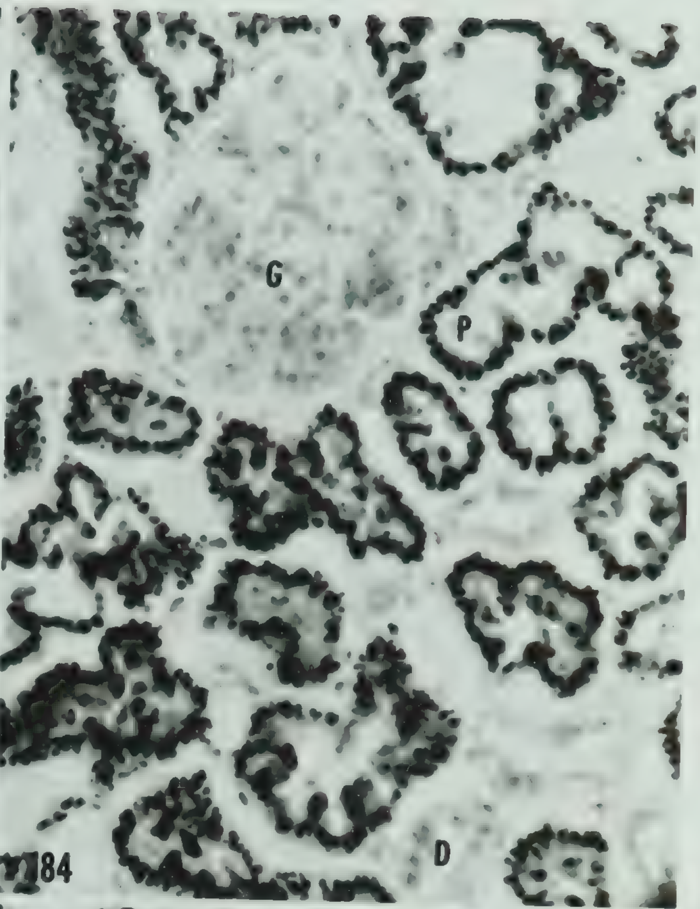

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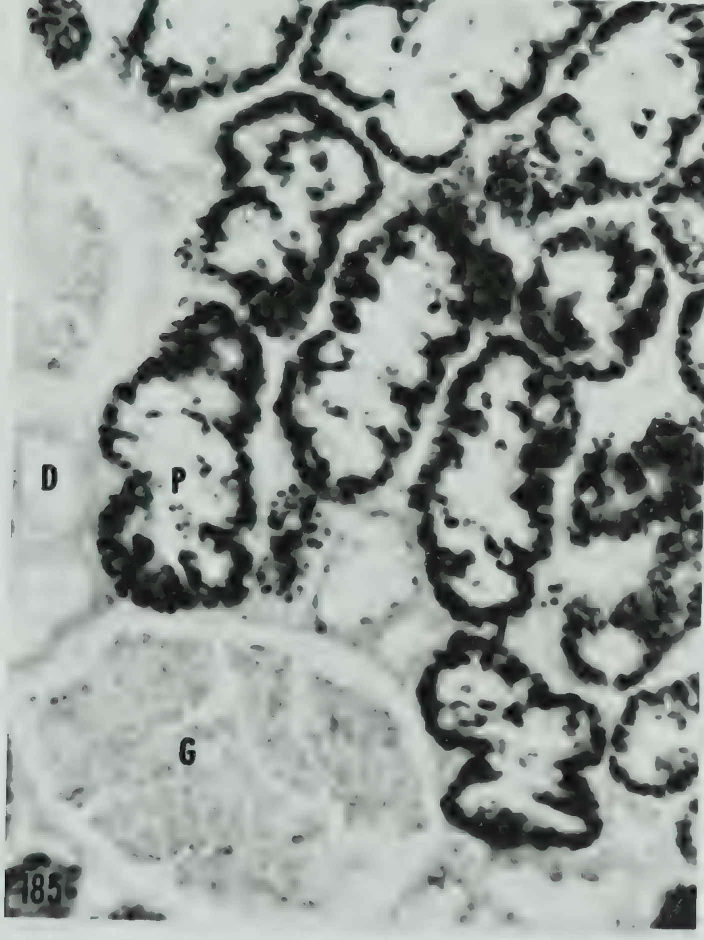

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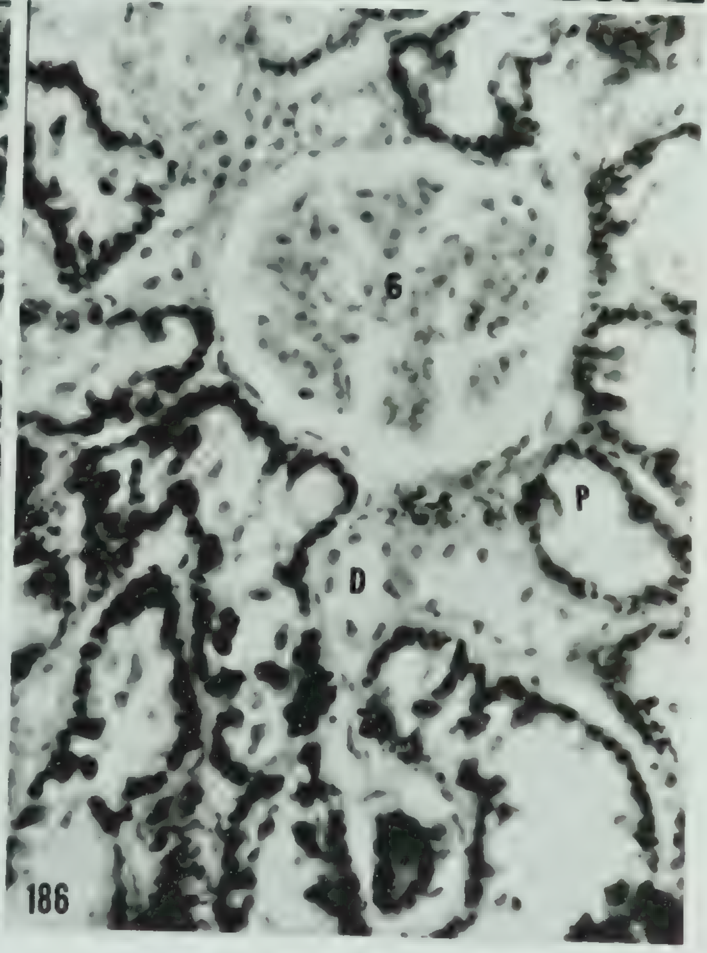

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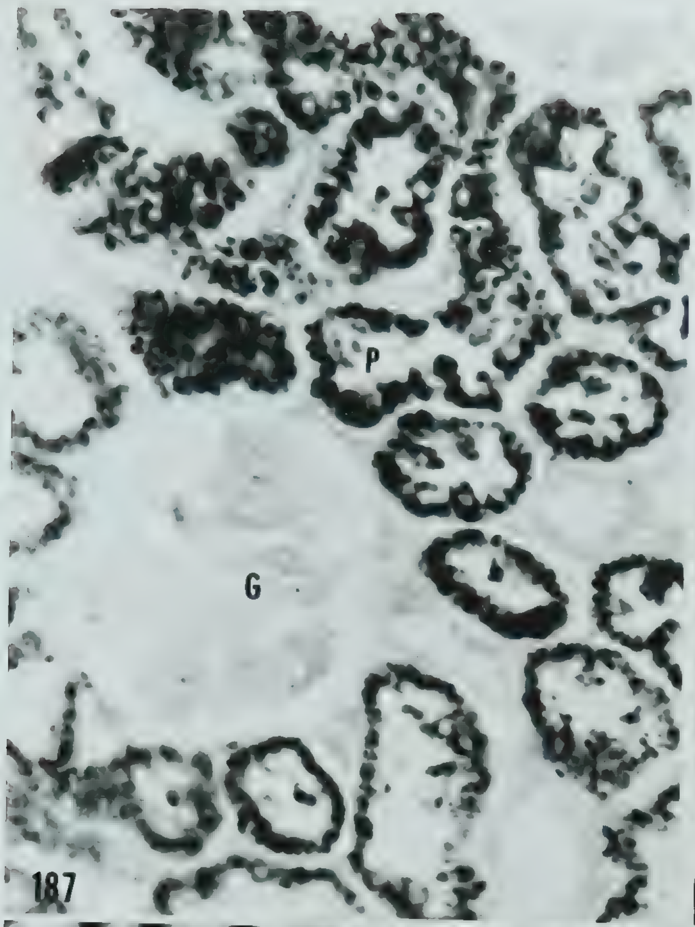
Fig. 187. Phenacetin experiment, (1.8 % dietary administration of phenacetin for 9 weeks), rat kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 188. Phenacetin experiment, (1.8 % dietary administration of phenacetin for 9 weeks), rat kidney, B-glycerophosphate phosphatase. 250 X.

Fig. 189. Normal rat liver, control for phenacetin experiment, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 190. Normal rat liver, control for phenacetin experiment, B-glycerophosphate phosphatase. 250 X.



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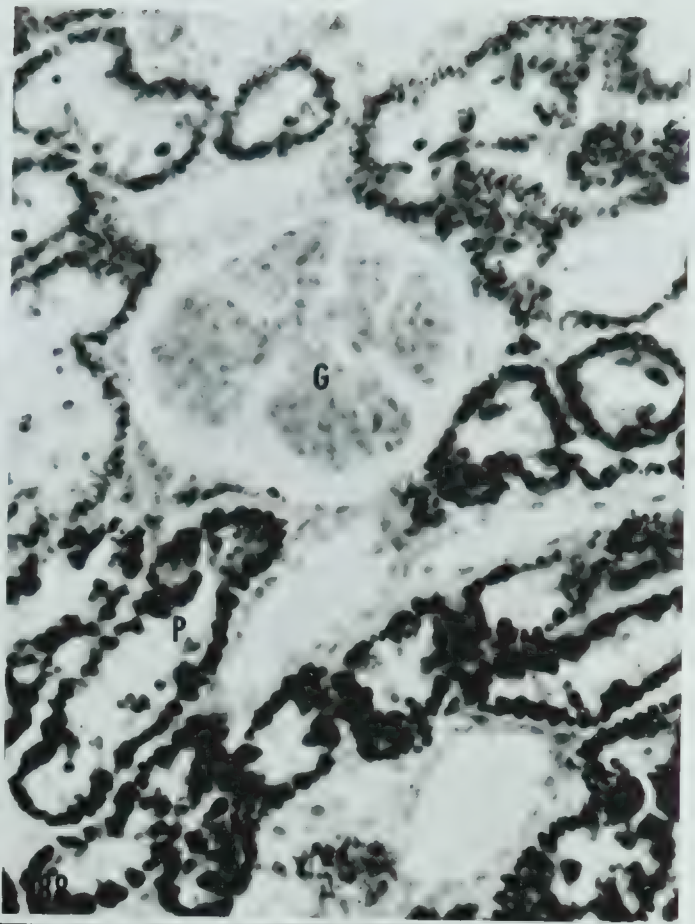

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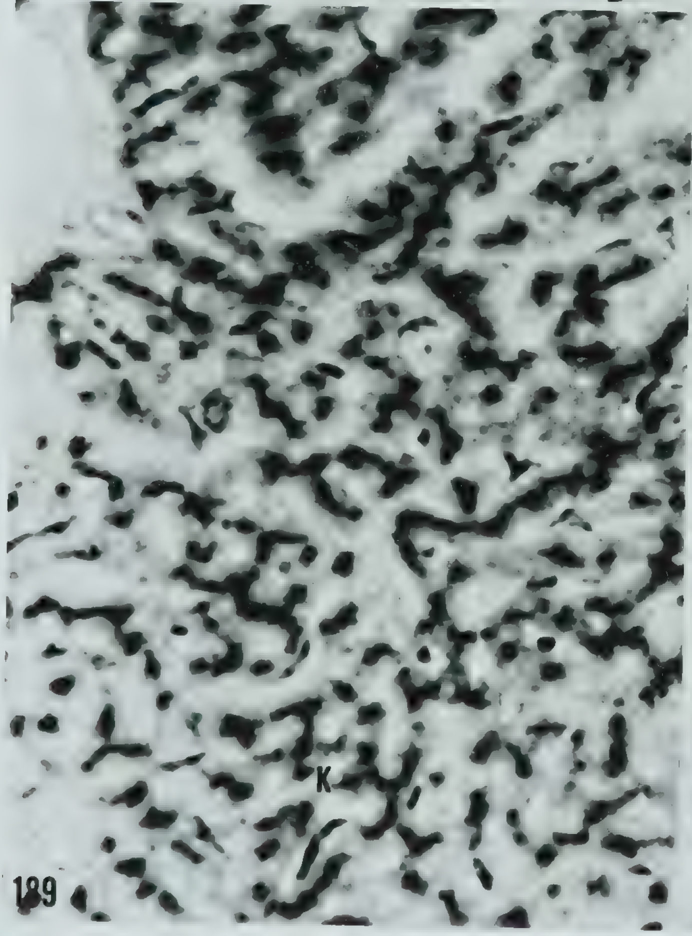

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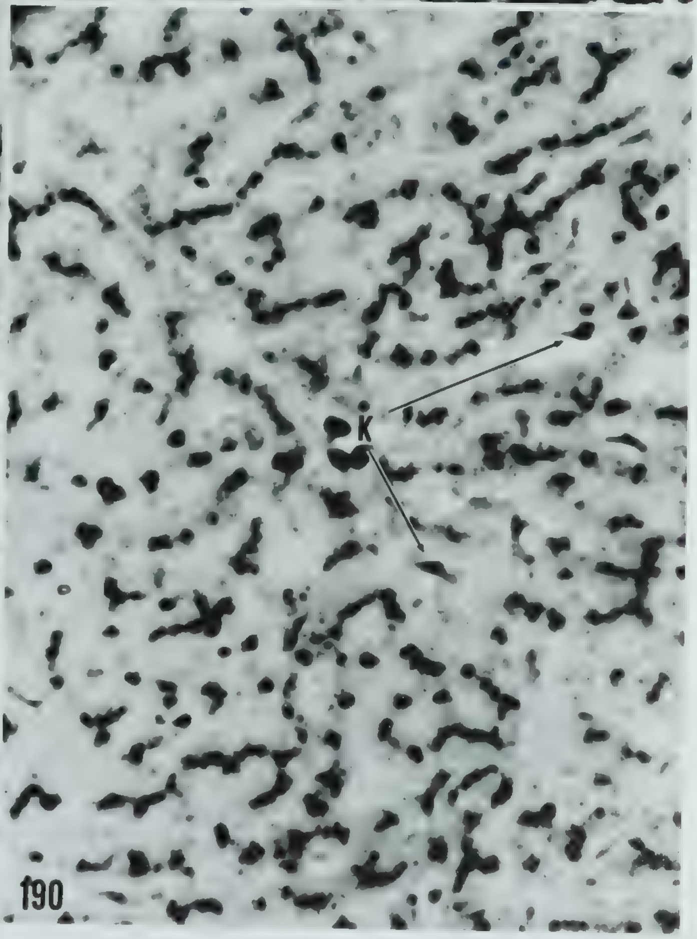

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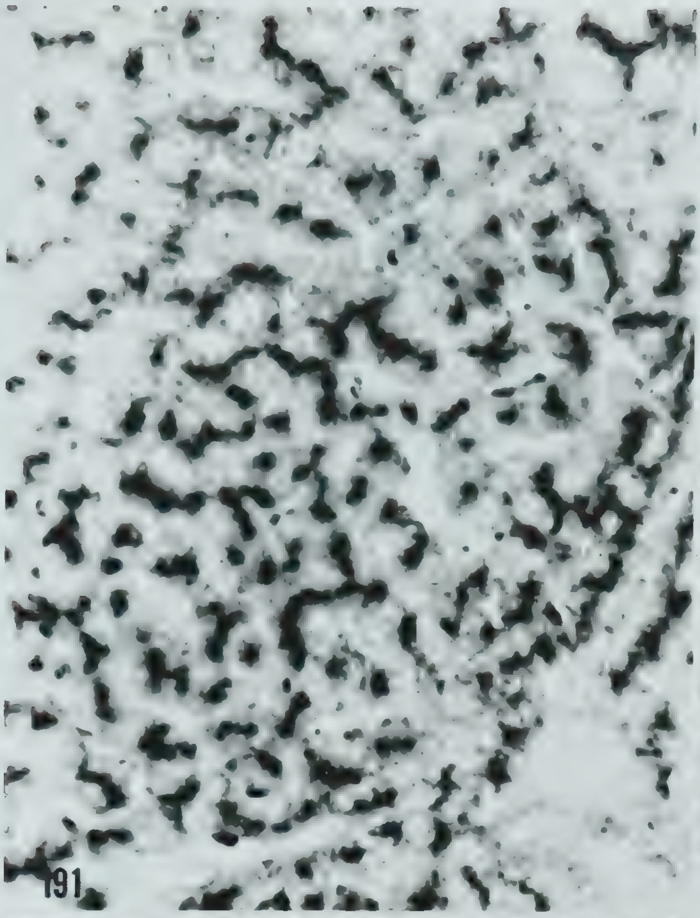
Fig. 191. Phenacetin experiment, (1.8 % dietary administration of phenacetin for 9 weeks), rat liver, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 192. Phenacetin experiment, (1.8 % dietary administration of phenacetin for 9 weeks), rat liver, B-glycerophosphate phosphatase. 250 X.

Fig. 193. Phenacetin experiment, (1.8 % dietary administration of phenacetin for 9 weeks), rat liver, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 194. Phenacetin experiment, (1.8 % dietary administration of phenacetin for 9 weeks), rat liver, B-glycerophosphate phosphatase. 250 X.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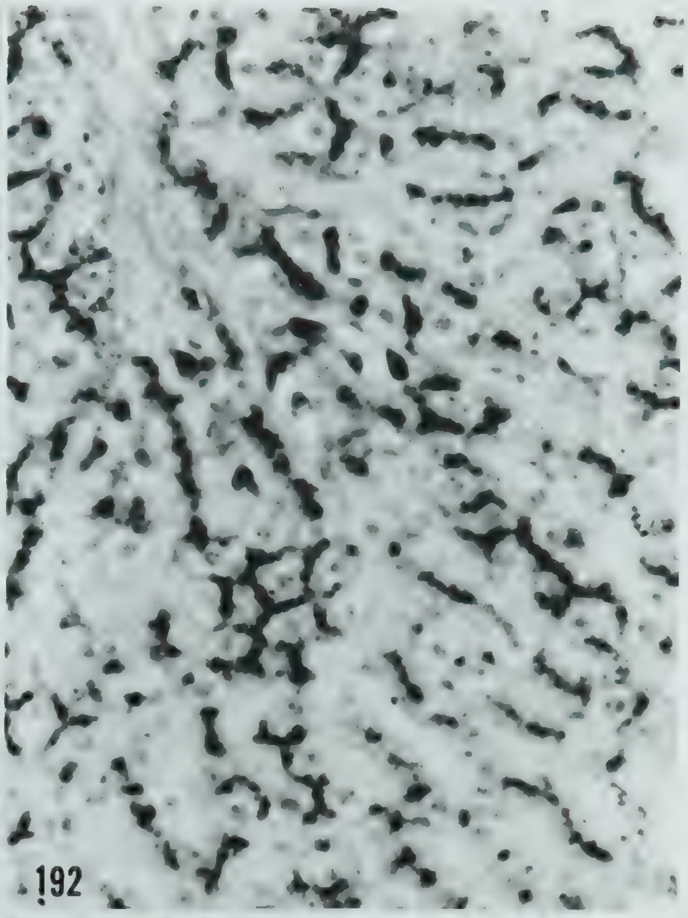
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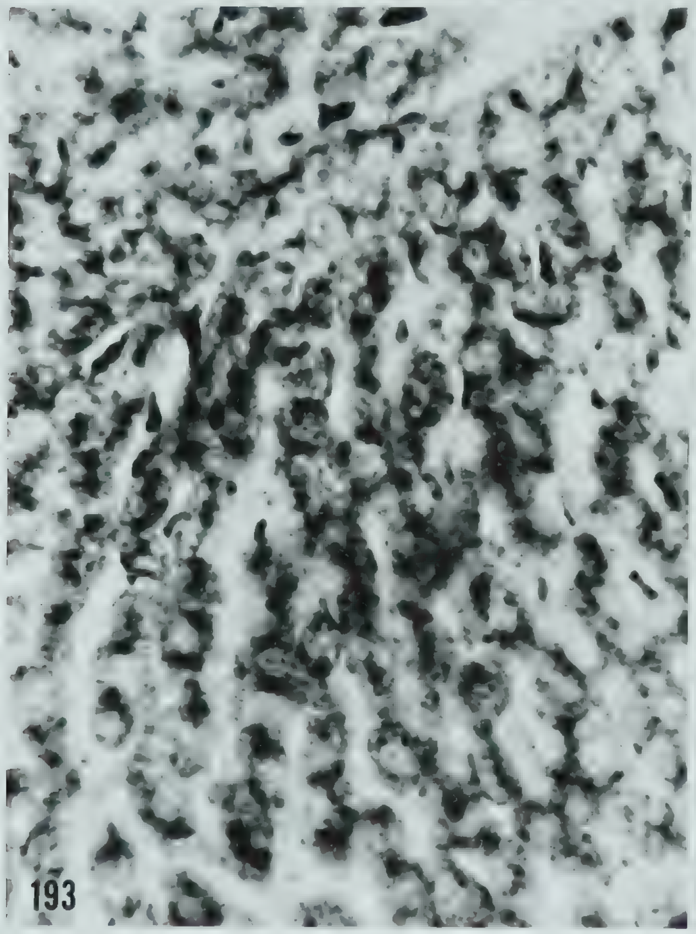
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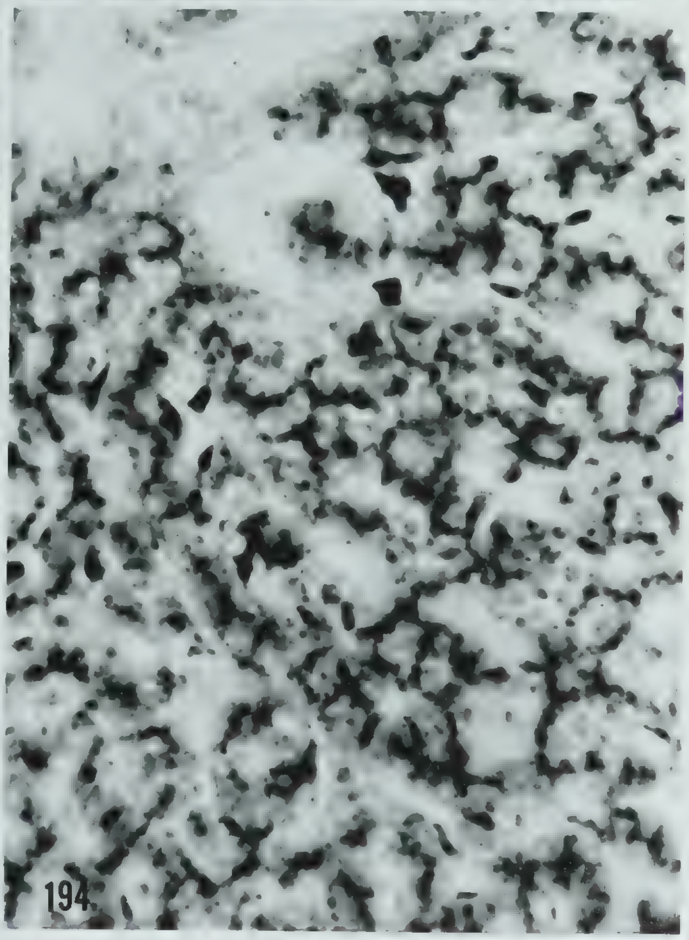
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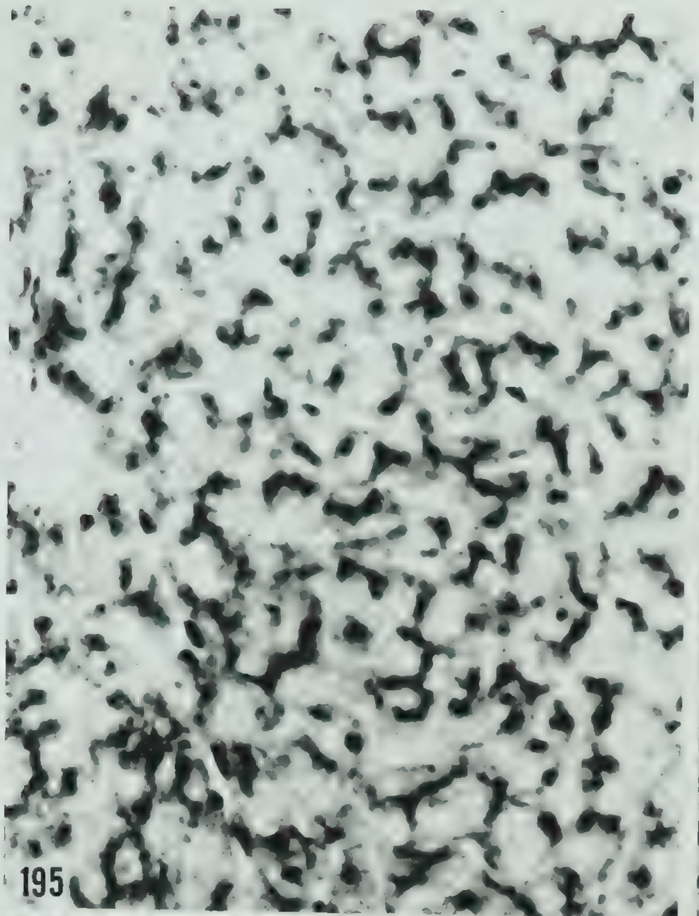
Fig. 195. Normal rat liver, control for cortisone experiment, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 196. Normal rat liver, control for cortisone experiment, B-glycerophosphate phosphatase. 250 X.

Fig. 197. Cortisone experiment, (subcutaneous administration of 10 mg. cortisone per day for 13 days), rat liver, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 198. Cortisone experiment, (subcutaneous administration of 10 mg. cortisone per day for 13 days), rat liver, B-glycerophosphate phosphatase. 250 X.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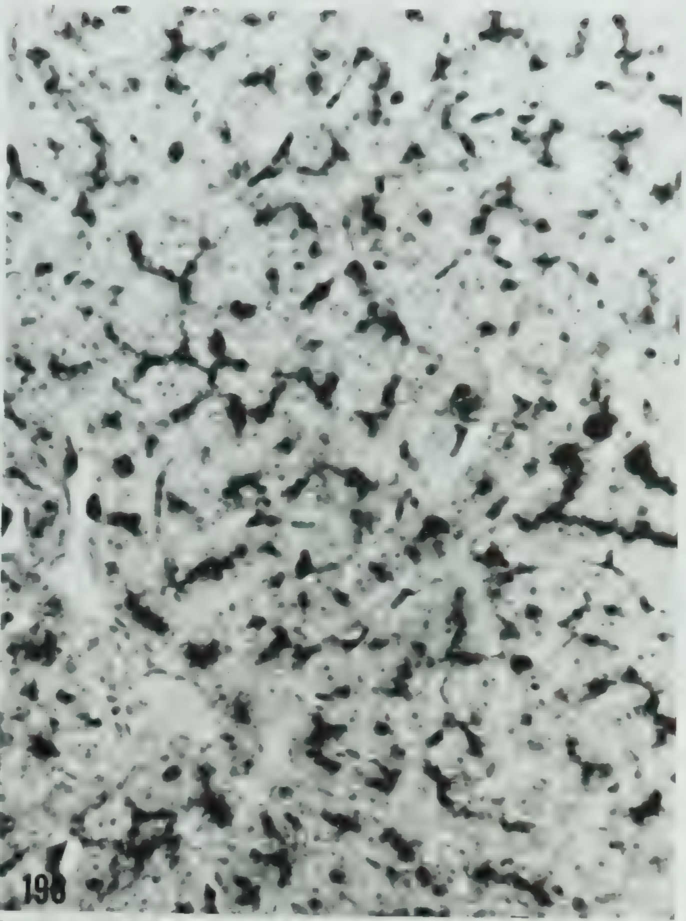
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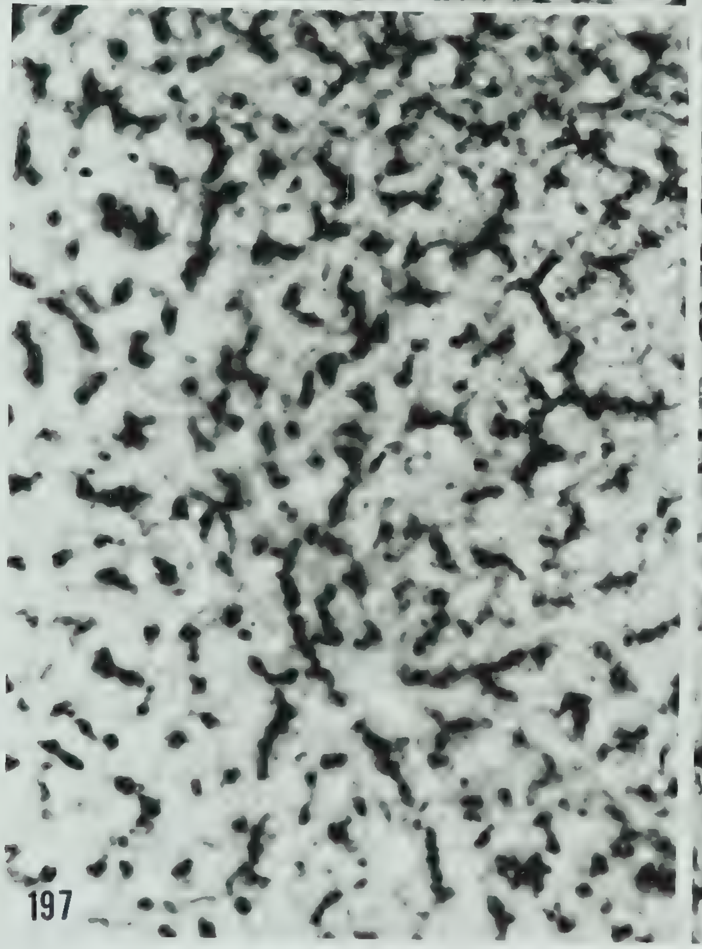
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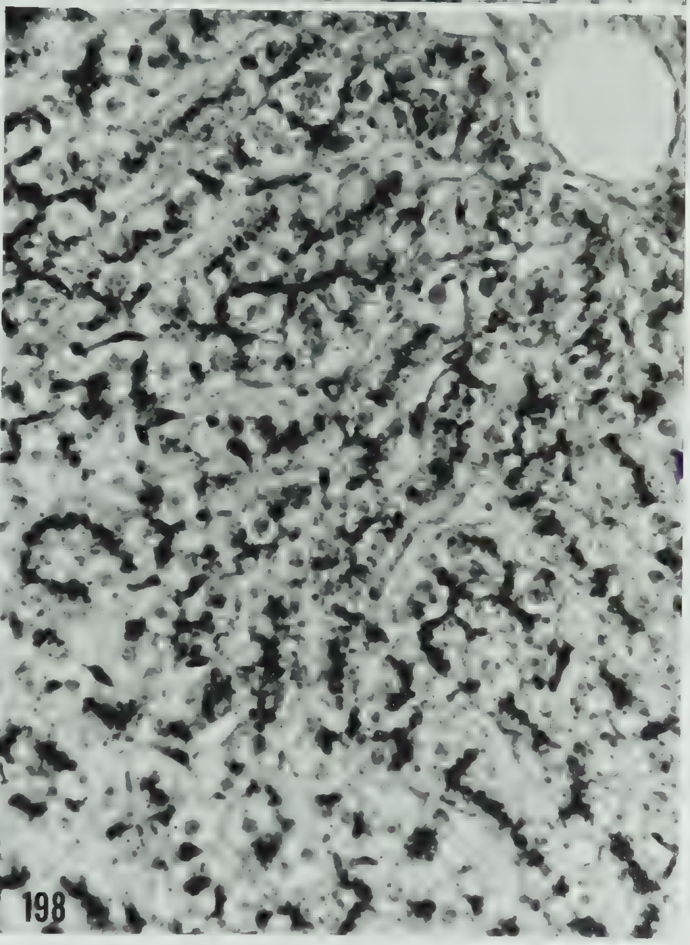
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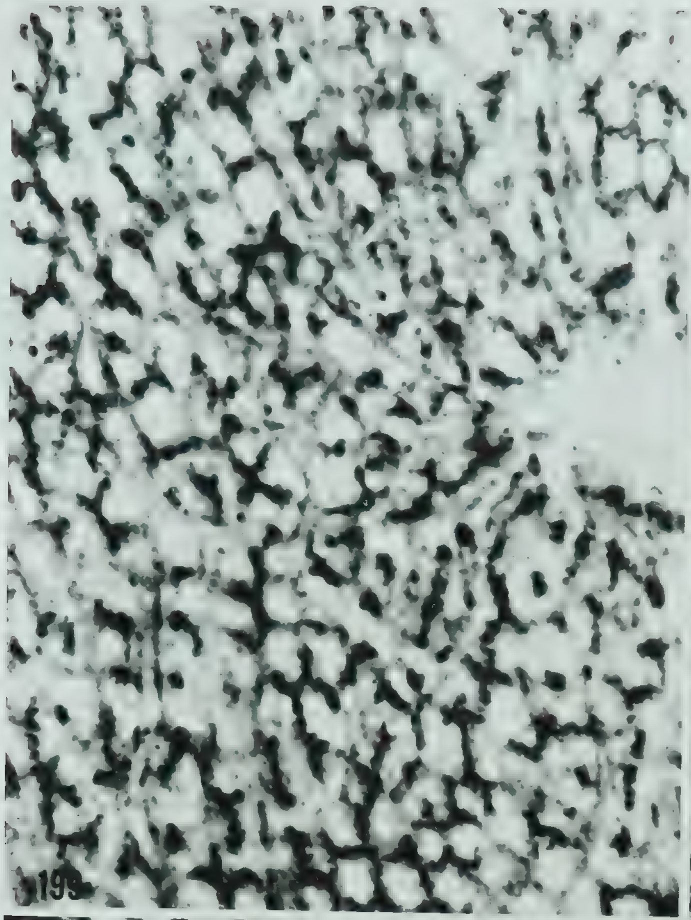
Fig. 199. Cortisone experiment, (subcutaneous administration of 10 mg. cortisone per day for 13 days), rat liver, Naphthol AS-TR phosphatase. 250 X.

Fig. 200. Cortisone experiment, (subcutaneous administration of 10 mg. cortisone per day for 13 days), rat liver, B-glycerophosphate phosphatase. 250 X.

Fig. 201. Normal rat kidney, control for cortisone experiment, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 202. Normal rat kidney, control for cortisone experiment, B-glycerophosphate phosphatase. 250 X.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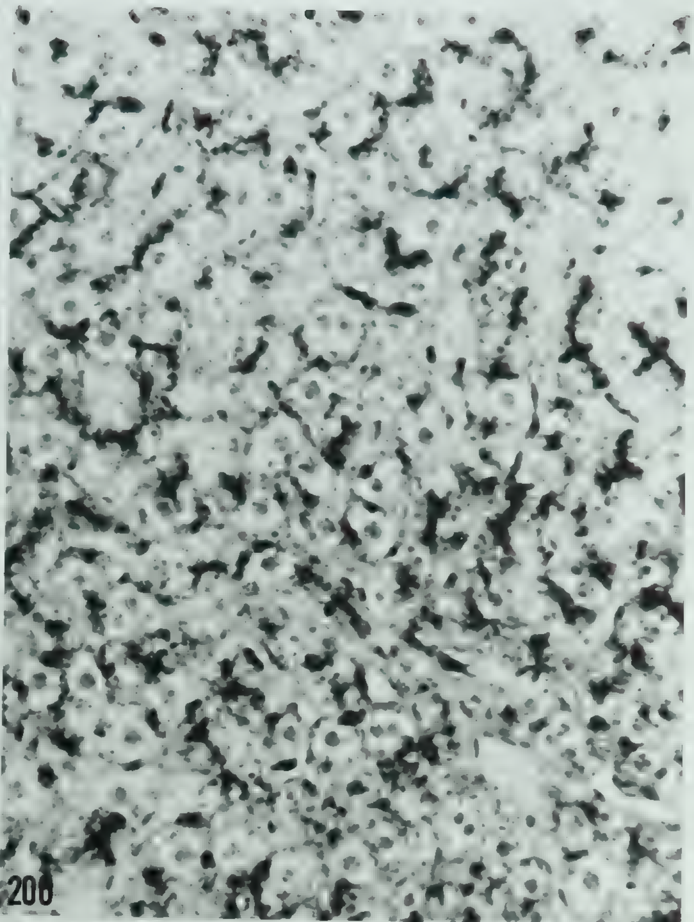
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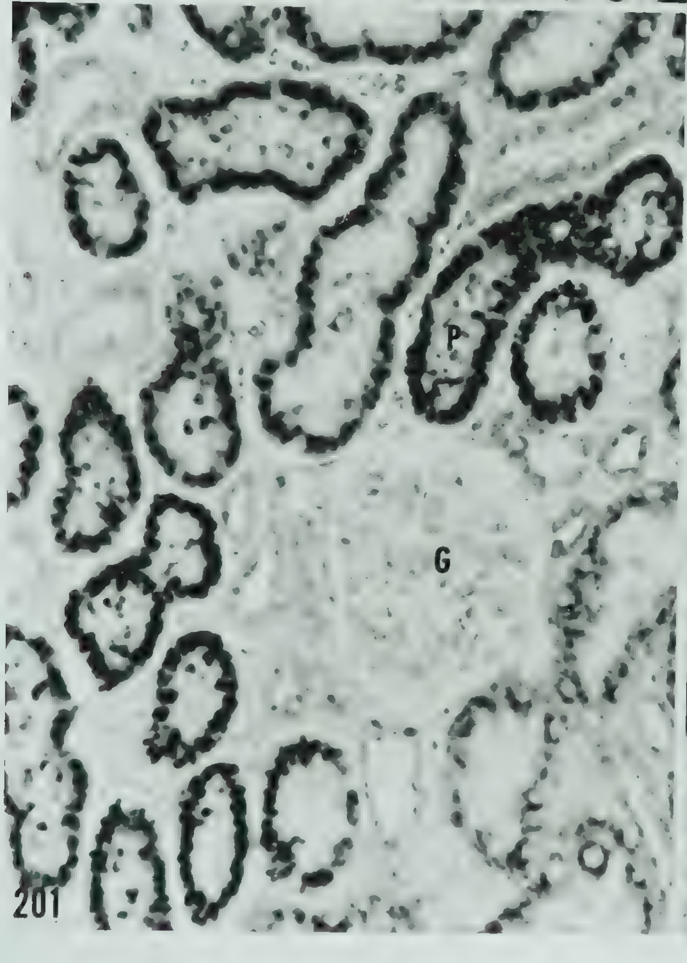

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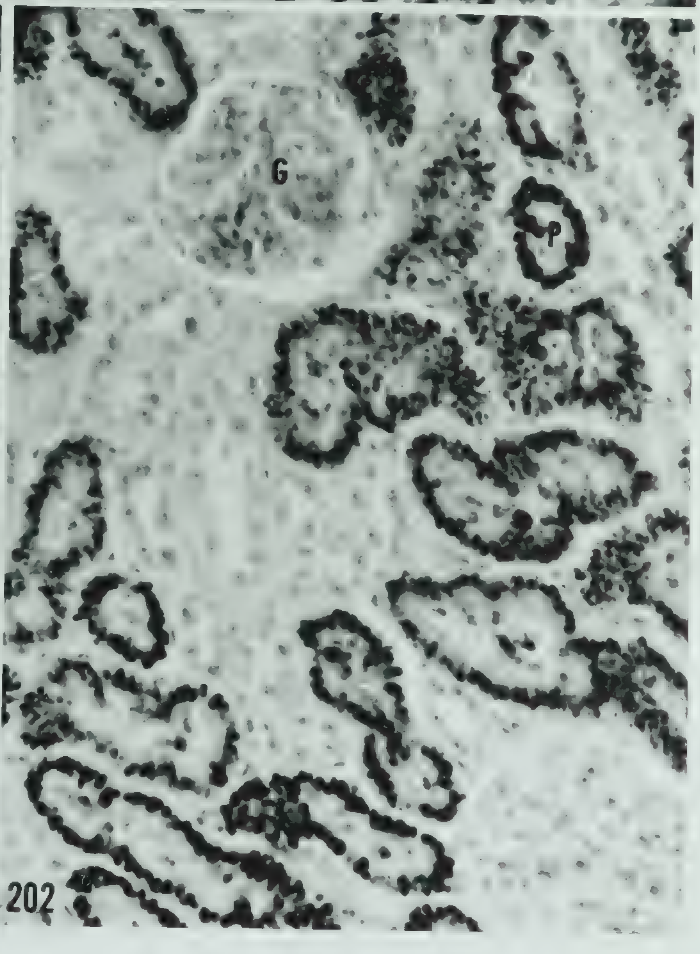

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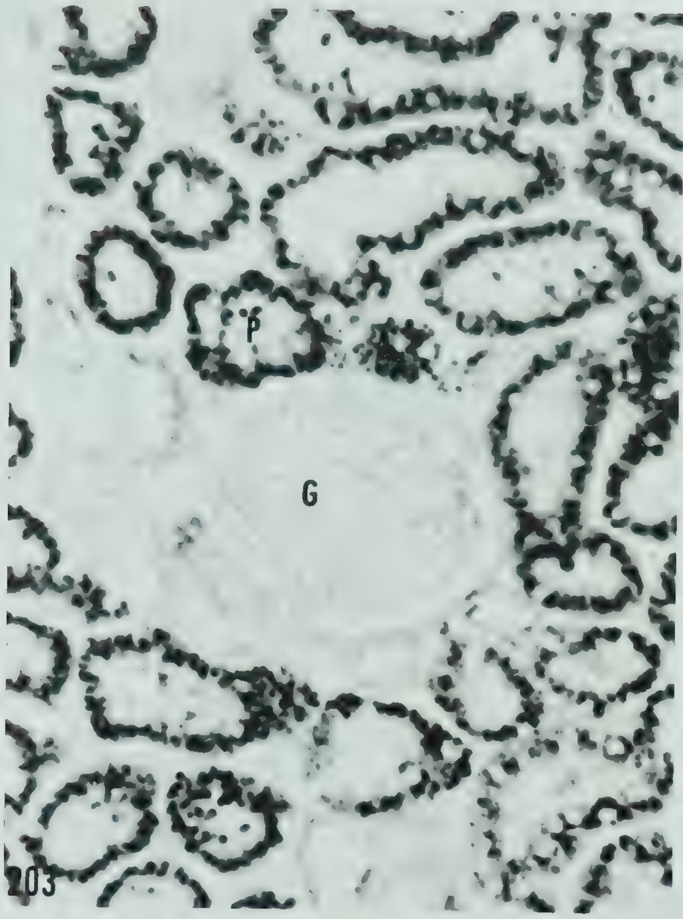
Fig. 203. Cortisone experiment, (subcutaneous administration of 10 mg. cortisone per day for 13 days), rat kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 204. Cortisone experiment, (subcutaneous administration of 10 mg. cortisone per day for 13 days), rat kidney, B-glycerophosphate phosphatase. 250 X.

Fig. 205. Cortisone experiment, (subcutaneous administration of 10 mg. cortisone per day for 13 days), rat kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 206. Cortisone experiment,(subcutaneous administration of 10 mg. cortisone per day for 13 days), rat kidney, B-glycerophosphate phosphatase. 250 X.



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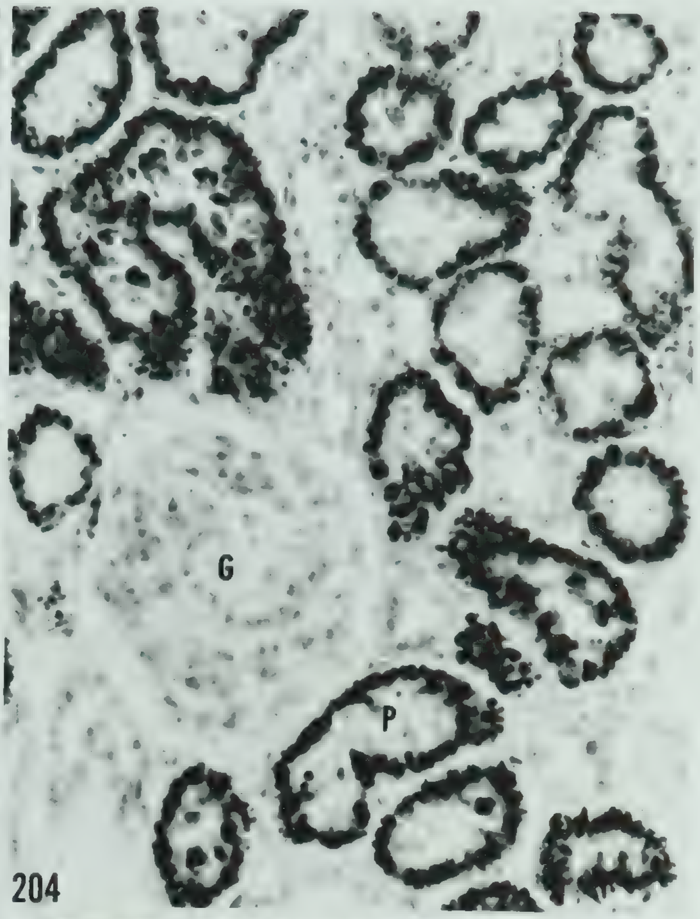

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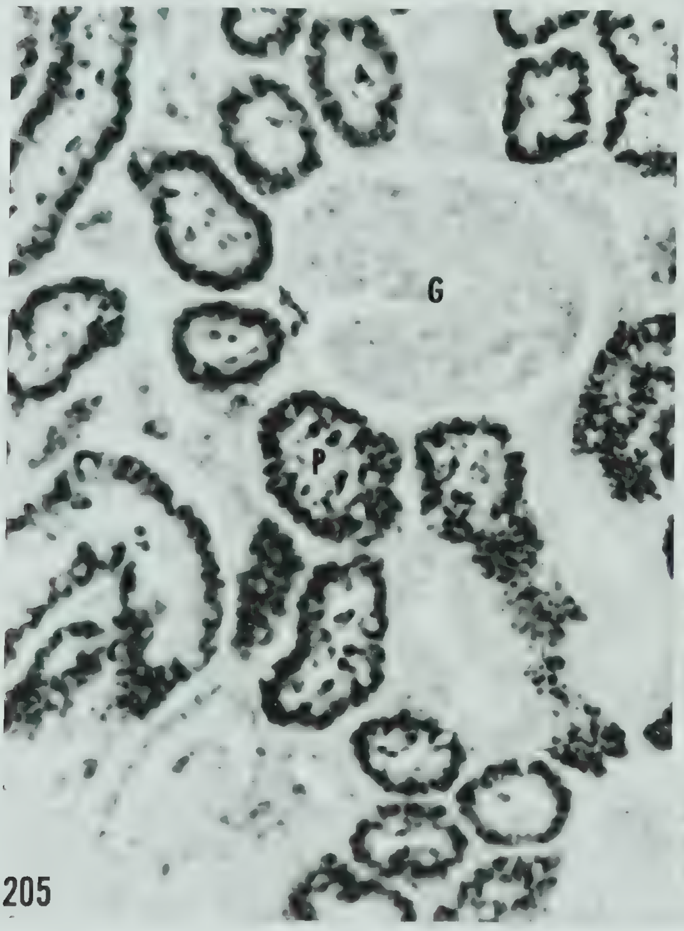

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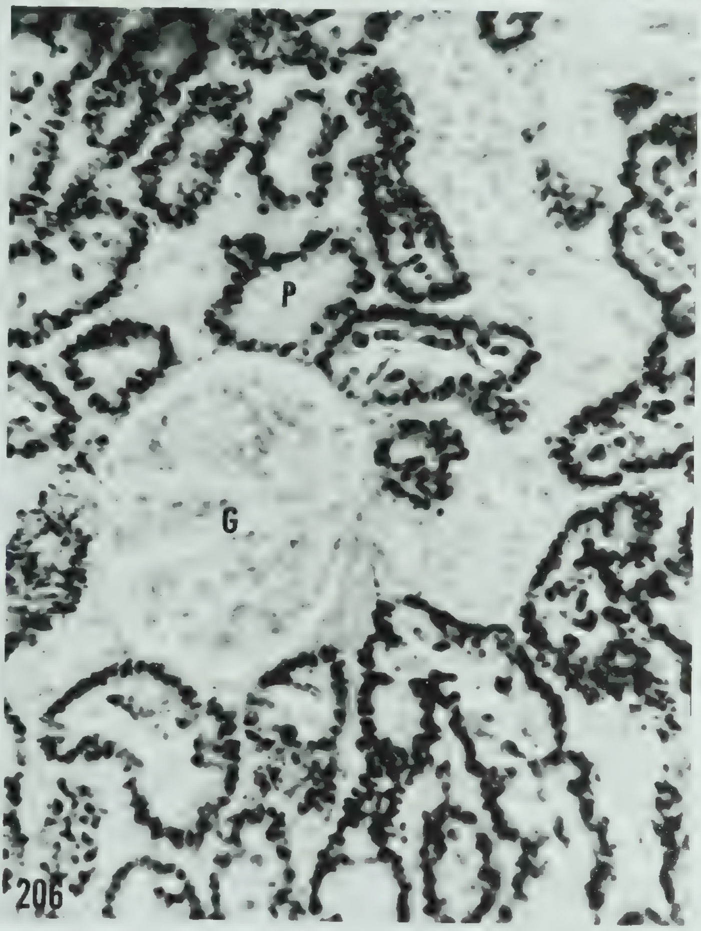

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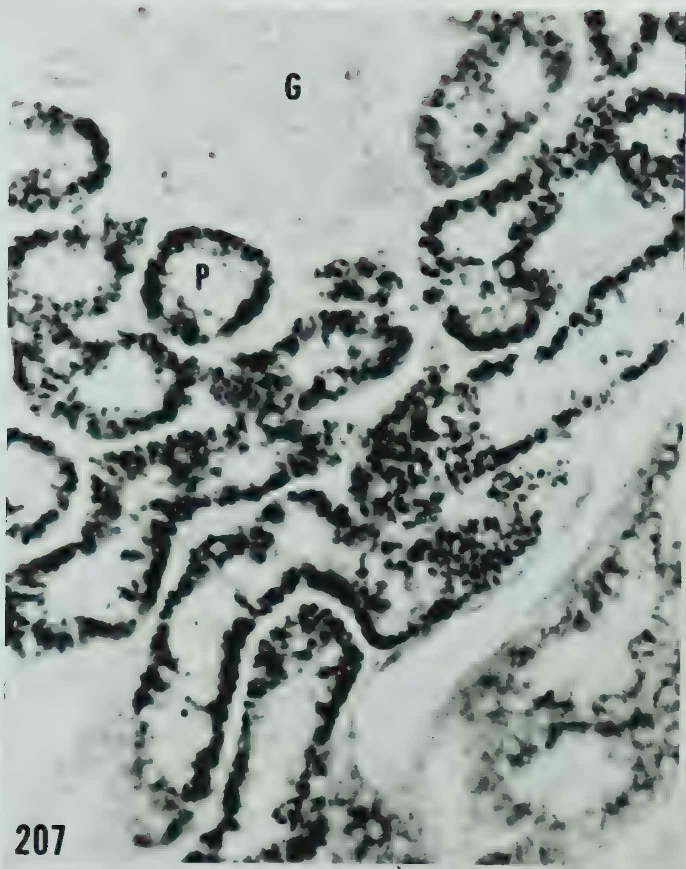
Fig. 207. Normal rat kidney, control for glucagon experiment, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 208. Normal rat liver, control for glucagon experiment, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 209. Normal rat kidney, control for glucagon experiment, B-glycerophosphate phosphatase. 250 X.

Fig. 210. Normal rat liver, control for glucagon experiment, B-glycerophosphate phosphatase. 250 X.



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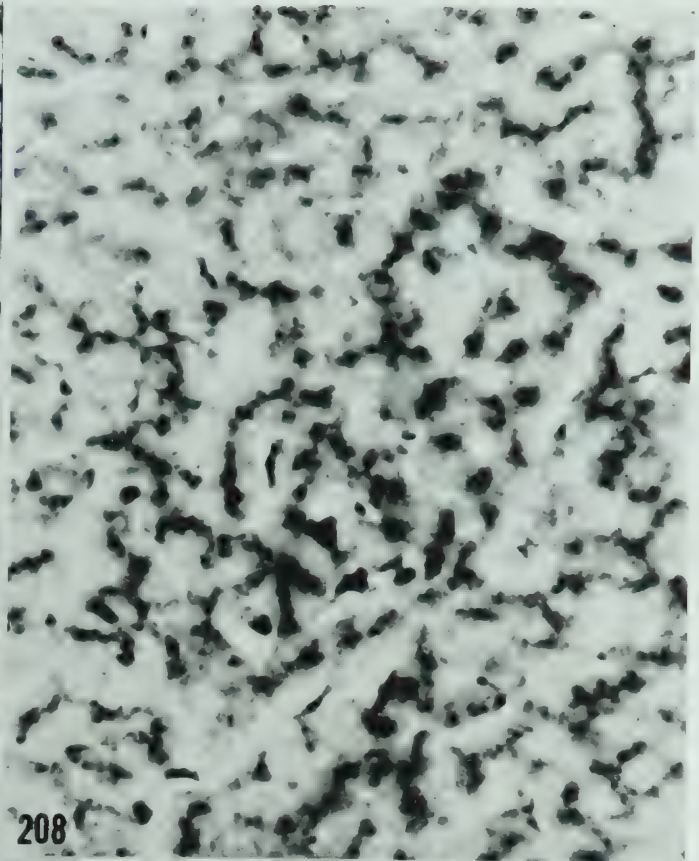

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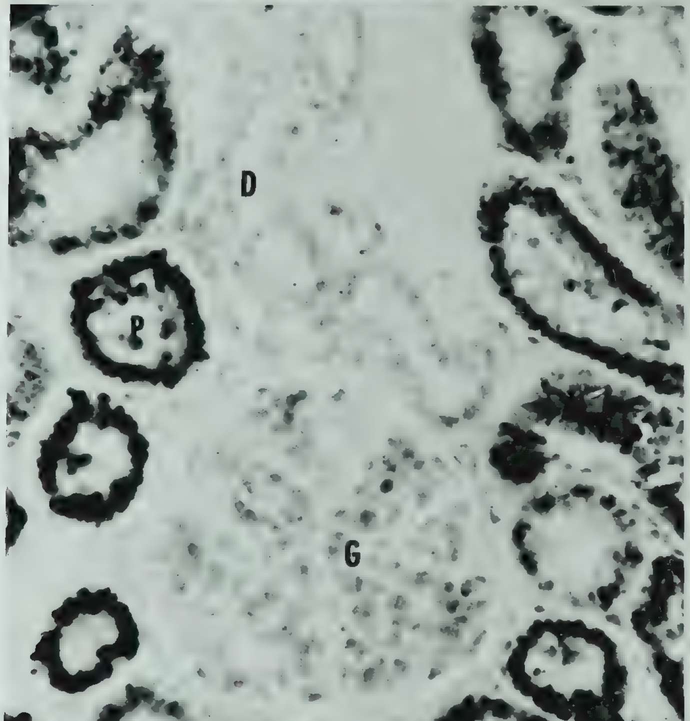

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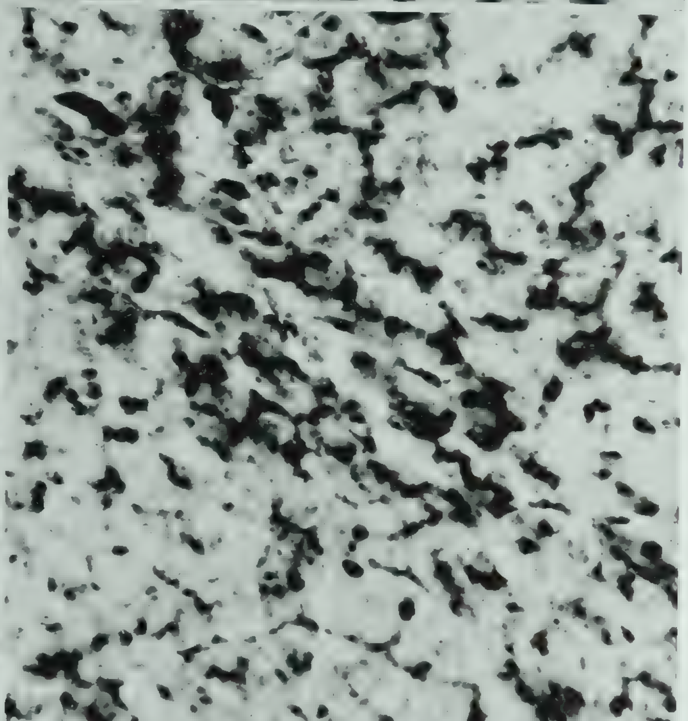

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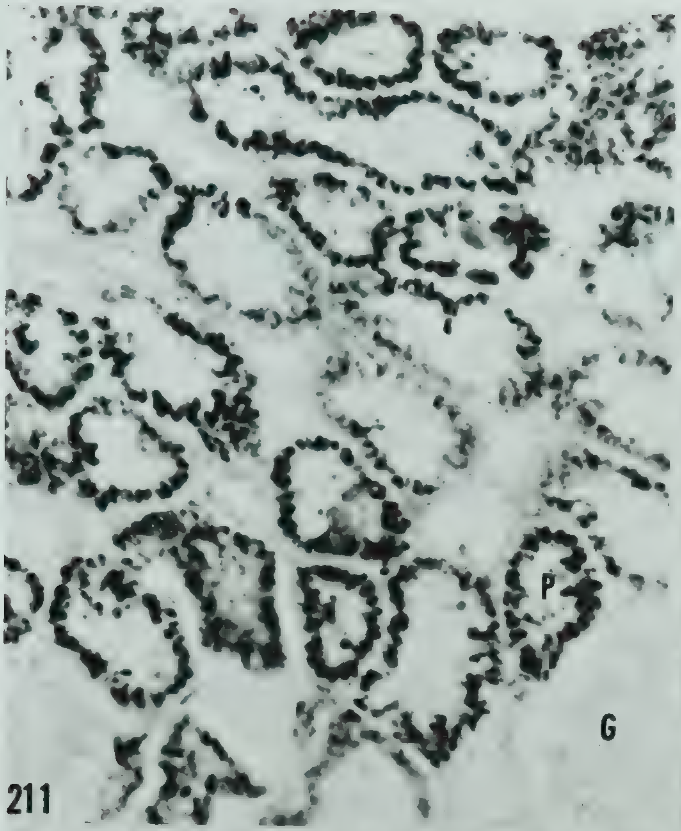
Fig. 211. Glucagon experiment, (intraperitoneal injection of 50 µg. glucagon per 100 g. body weight), rat kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 212. Glucagon experiment, (intraperitoneal injection of 50 µg. glucagon per 100 g. body weight), rat liver, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 213. Glucagon experiment, (intraperitoneal injection of 50 µg. glucagon per 100 g. body weight), rat kidney, B-glycerophosphate phosphatase. 250 X.

Fig. 214. Glucagon experiment, (intraperitoneal injection of 50 µg. glucagon per 100 g. body weight), rat liver, B-glycerophosphate phosphatase. 250 X.



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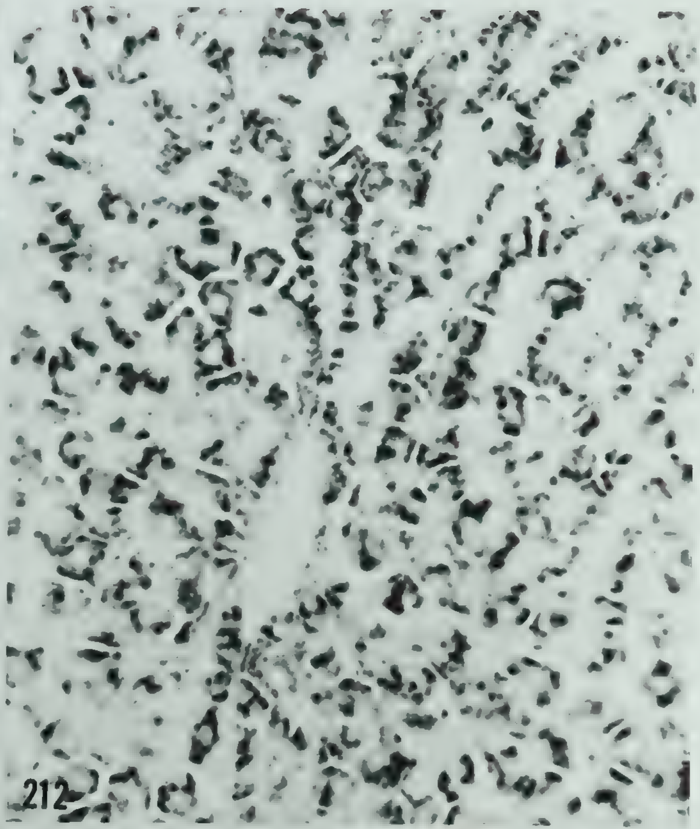

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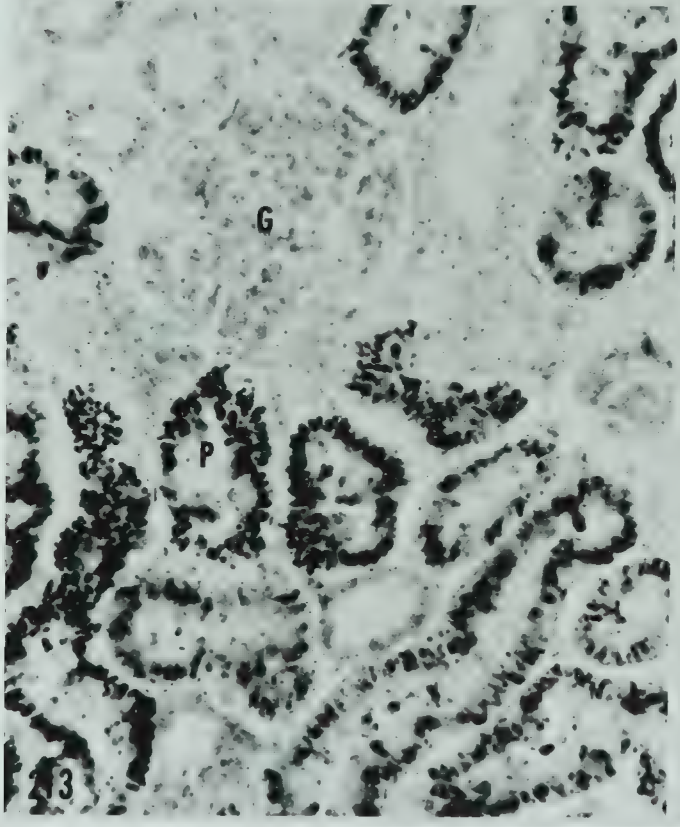

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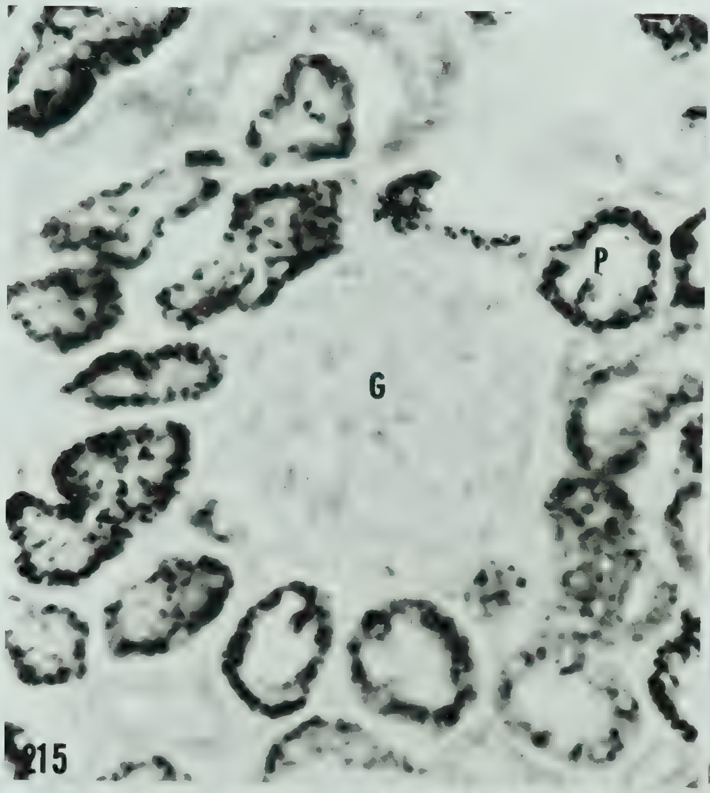
Fig. 215. Phosphorus experiment, (1.8 mg. yellow phosphorus suspended in mineral oil administered by stomach tube), rat kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 216. Phosphorus experiment, (1.8 mg. yellow phosphorus suspended in mineral oil administered by stomach tube), rat liver, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 217. Phosphorus experiment, (1.8 mg. yellow phosphorus suspended in mineral oil administered by stomach tube), rat kidney. B-glycerophosphate phosphatase. 250 X.

Fig. 218. Phosphorus experiment, (1.8 mg. yellow phosphorus suspended in mineral oil administered by stomach tube), rat liver, B-glycerophosphate phosphatase. 250 X.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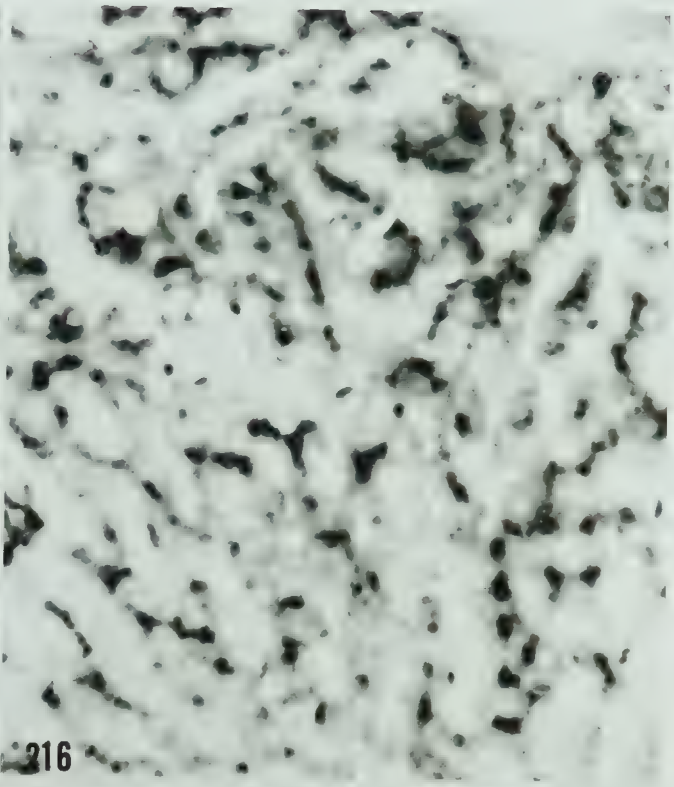
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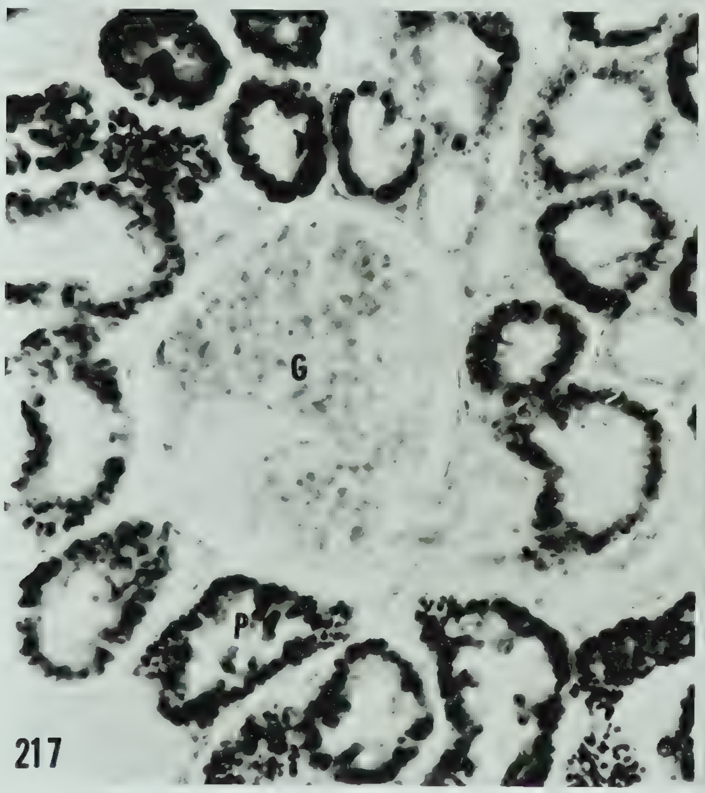
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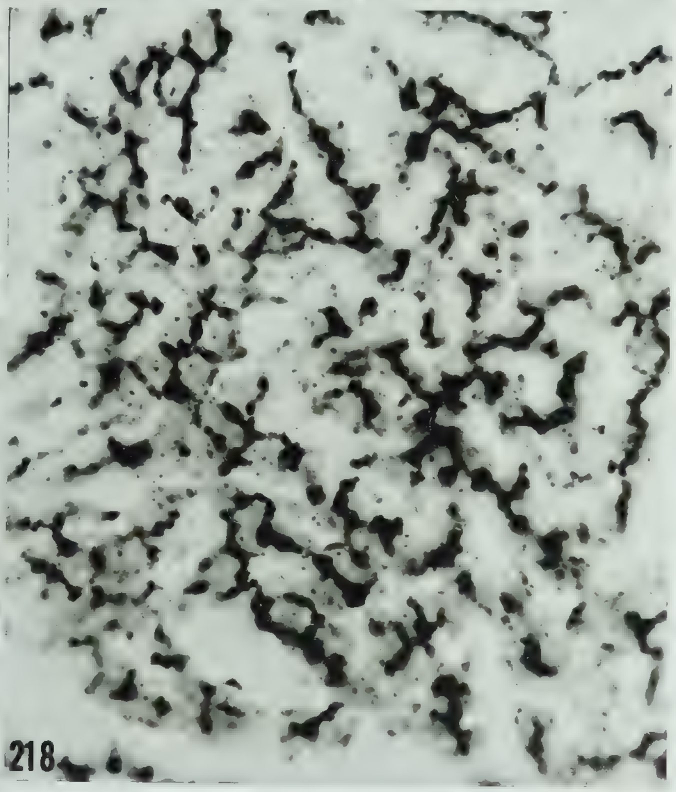
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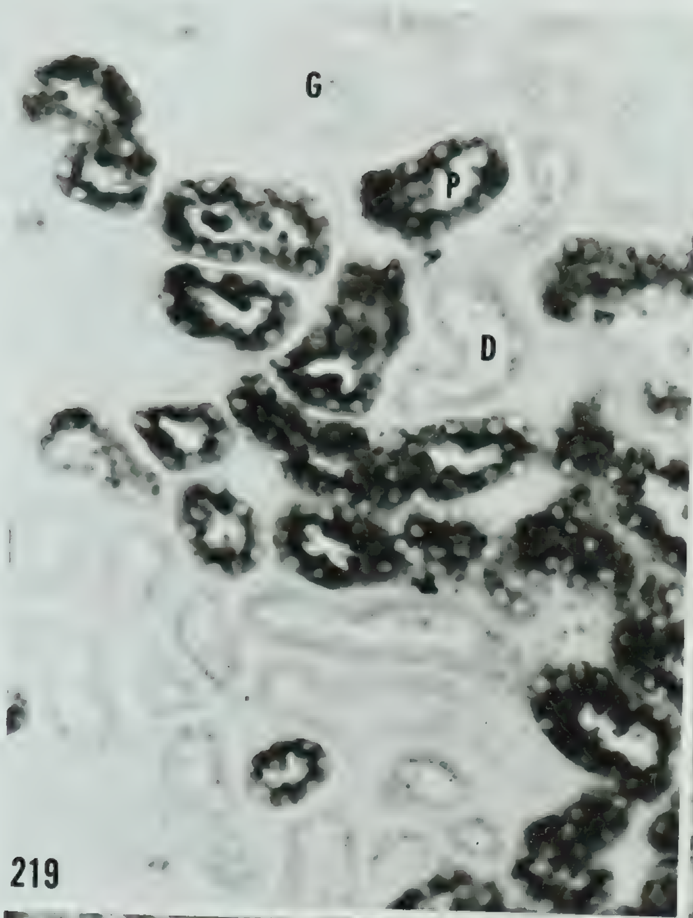
Fig. 219. Normal mouse kidney, control for testosterone propionate experiment, B-glucuronidase. 250 X.

Fig. 220. Normal mouse liver, control for testosterone propionate experiment, B-glucuronidase. 250 X.

Fig. 221. Normal mouse kidney, olive oil control for estrone experiment, B-glucuronidase. 250 X.

Fig. 222. Normal mouse liver, olive oil control for estrone experiment, B-glucuronidase. 250 X.



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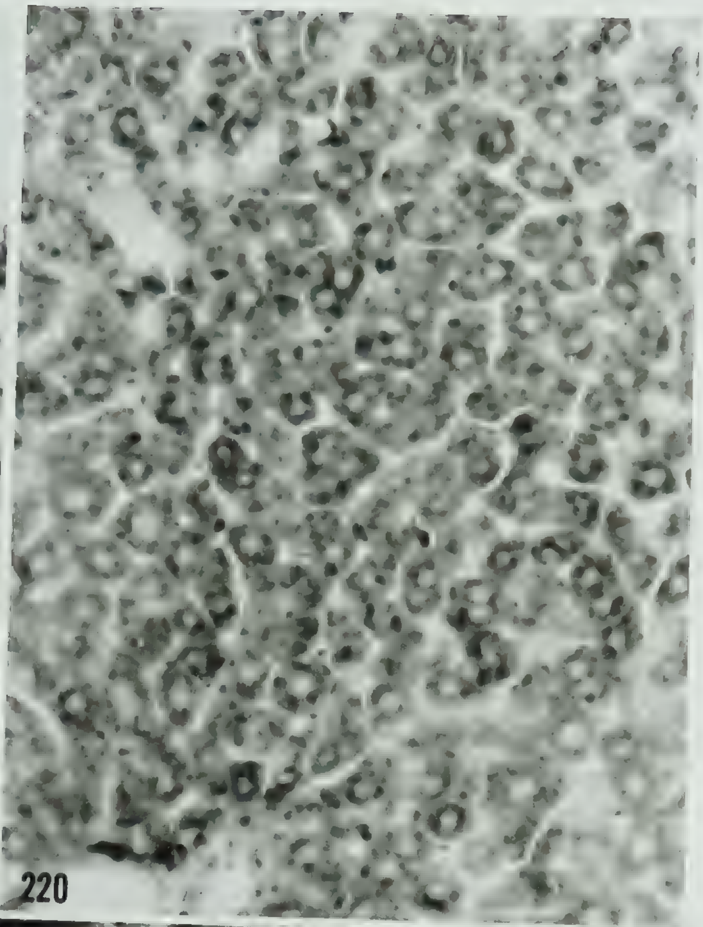

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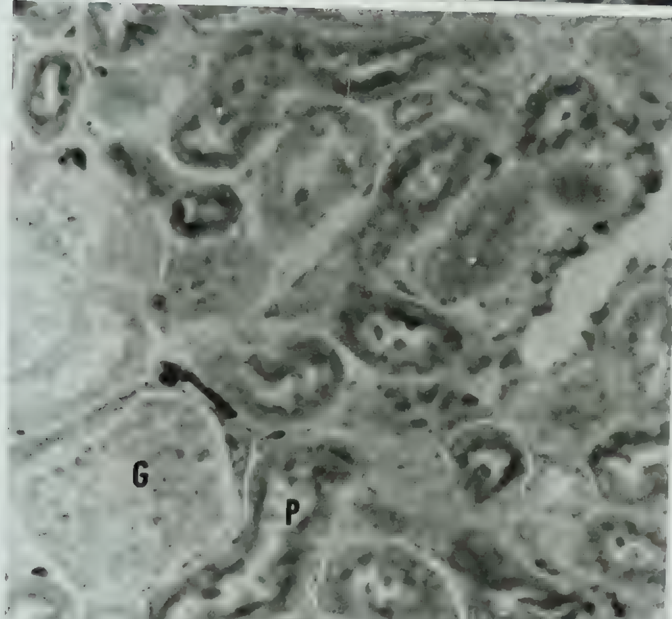

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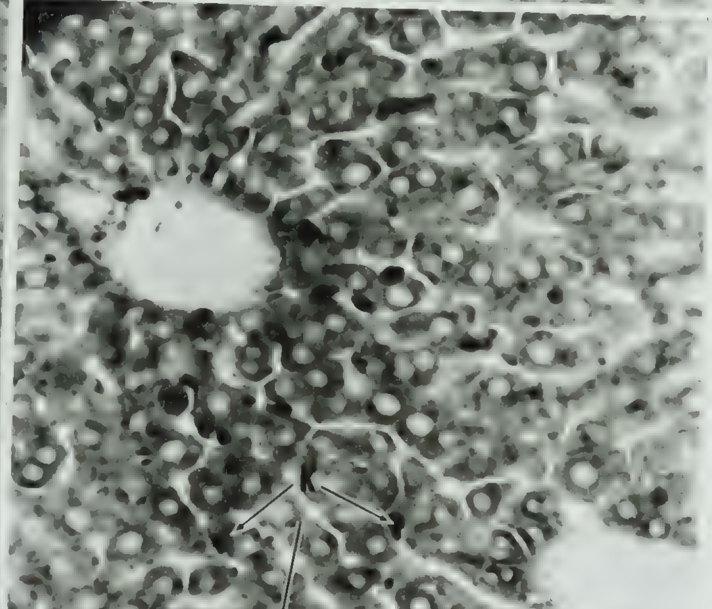

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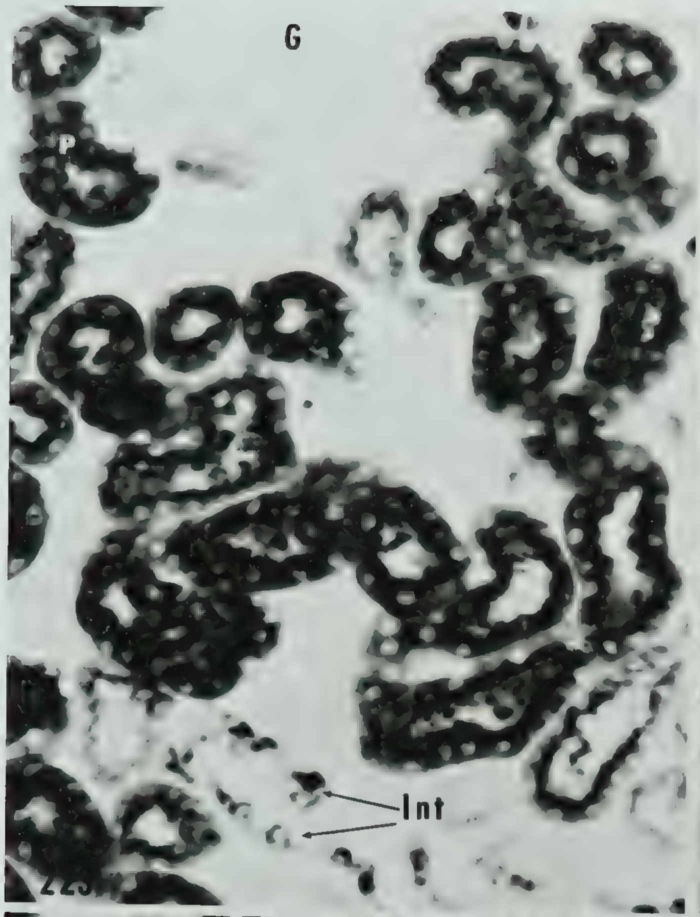
Fig. 223. Testosterone propionate experiment, (seven subcutaneous injections on alternate days of 1 mg. testosterone propionate dissolved in 0.1 ml. olive oil), mouse kidney, B-glucuronidase. 250 X.

Fig. 224. Testosterone propionate experiment, (seven subcutaneous injections on alternate days of 1 mg. testosterone propionate dissolved in 0.1 ml. olive oil), mouse liver, B-glucuronidase. 250 X.

Fig. 225. Testosterone propionate experiment, (seven subcutaneous injections on alternate days of 1 mg. testosterone propionate dissolved in 0.1 ml. olive oil), mouse kidney, B-glucuronidase. 250 X.

Fig. 226. Testosterone propionate experiment, (seven subcutaneous injections on alternate days of 1 mg. testosterone propionate dissolved in 0.1 ml. olive oil), mouse liver, B-glucuronidase. 250 X.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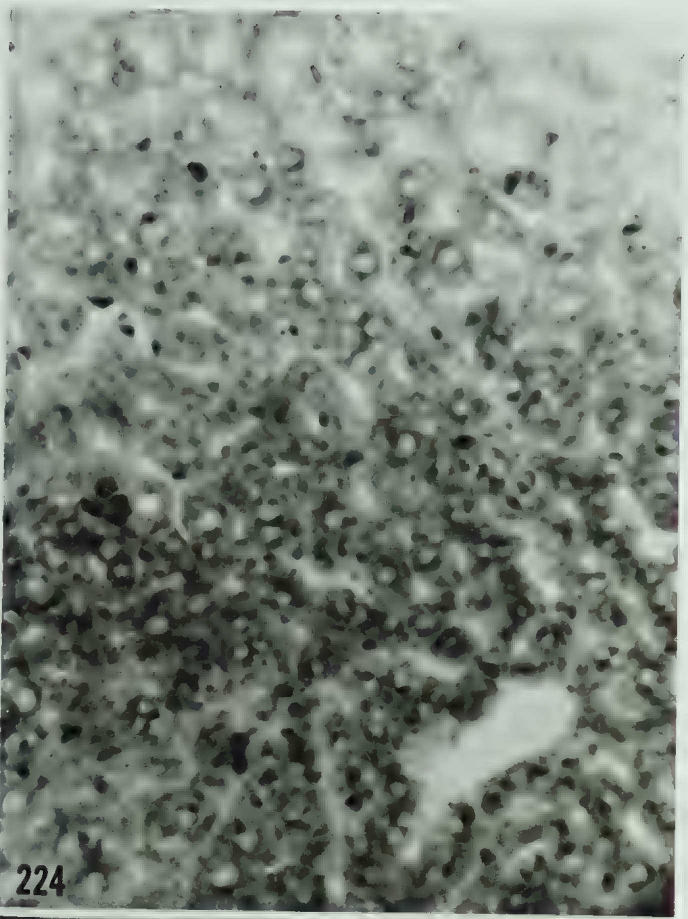
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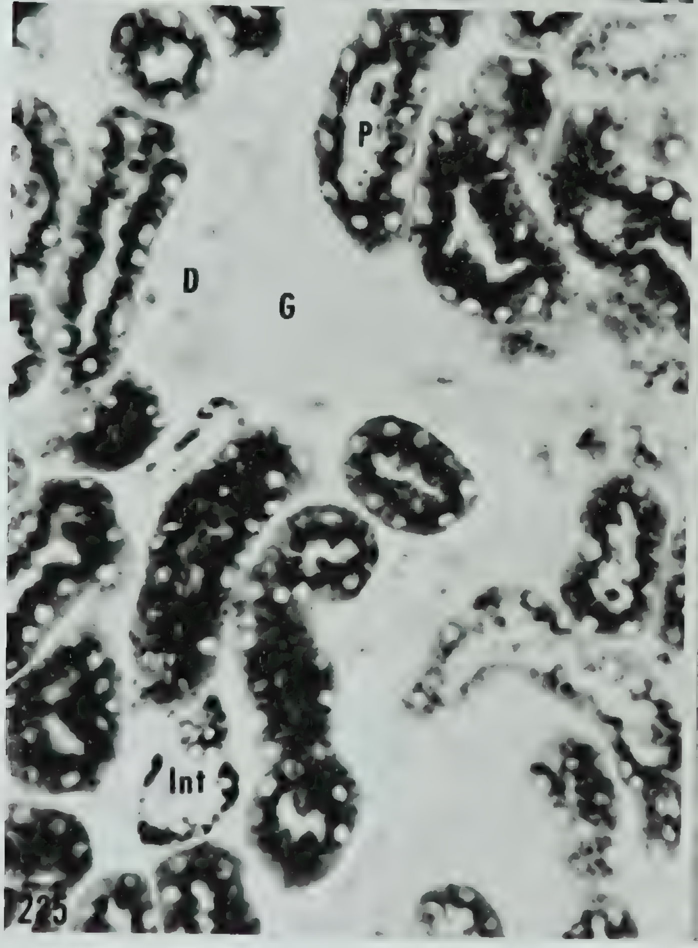
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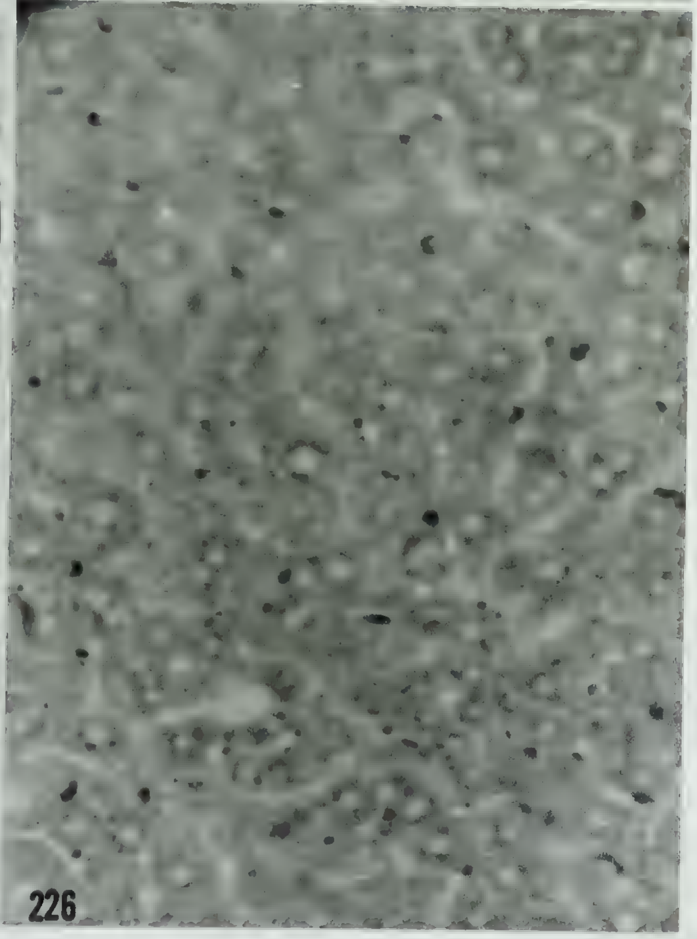
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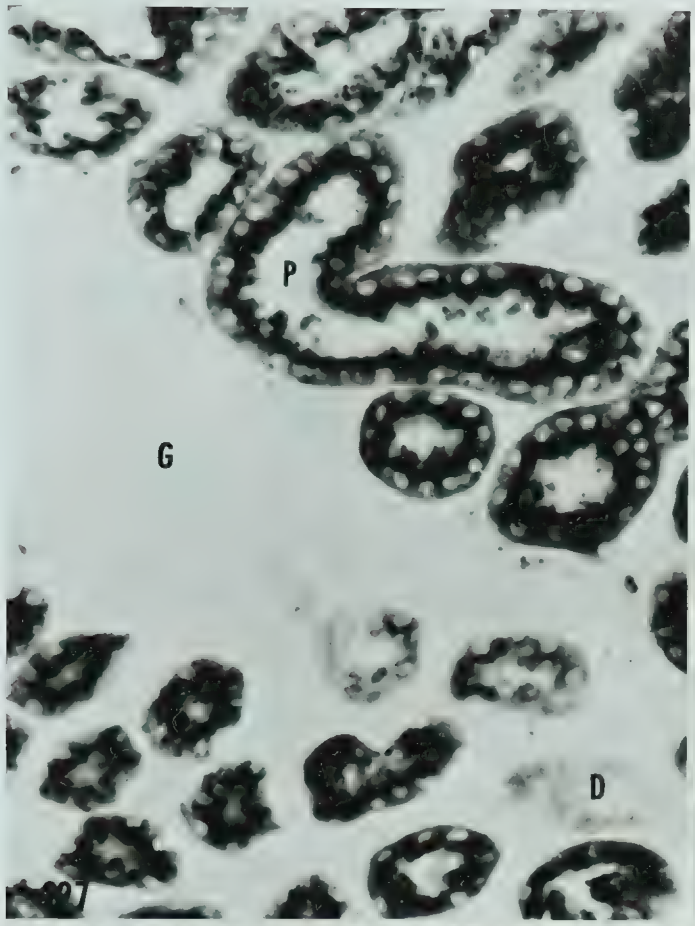
Fig. 227. Testosterone propionate experiment, (seven subcutaneous injections on alternate days of 1 mg. testosterone propionate dissolved in 0.1 ml. olive oil), mouse kidney, B-glucuronidase. 250 X.

Fig. 228. Testosterone propionate experiment, (seven subcutaneous injections on alternate days of 1 mg. testosterone propionate dissolved in 0.1 ml. olive oil), mouse liver, B-glucuronidase. 250 X.

Fig. 229. Estrone experiment, (subcutaneous injection of 0.05 mg. estrone dissolved in 0.25 ml. olive oil), mouse kidney, B-glucuronidase. 250 X.

Fig. 230. Estrone experiment, (subcutaneous injection of 0.05 mg. estrone dissolved in 0.25 ml. olive oil), mouse liver, B-glucuronidase. 250 X.



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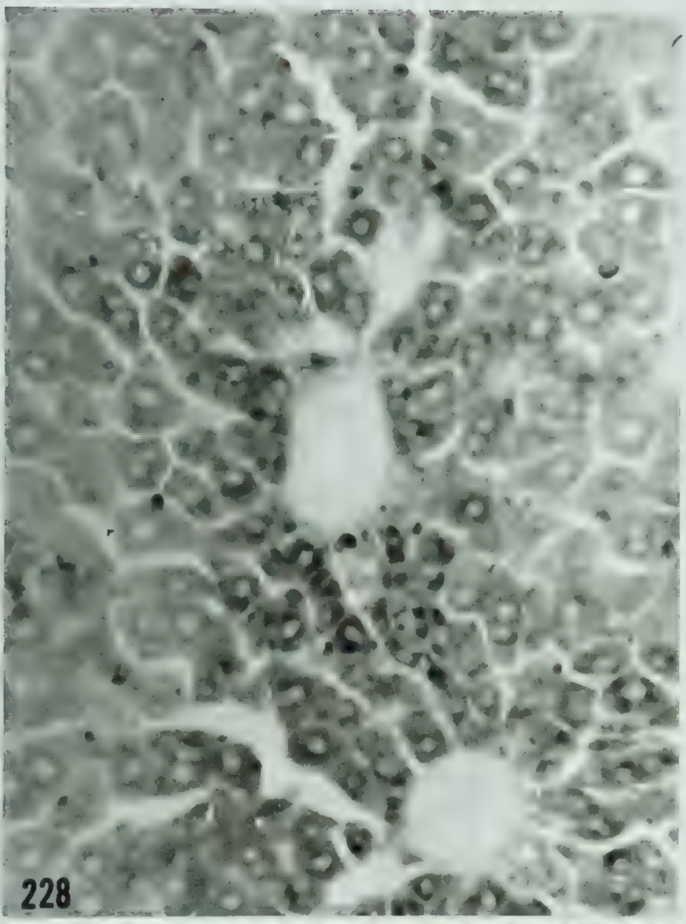
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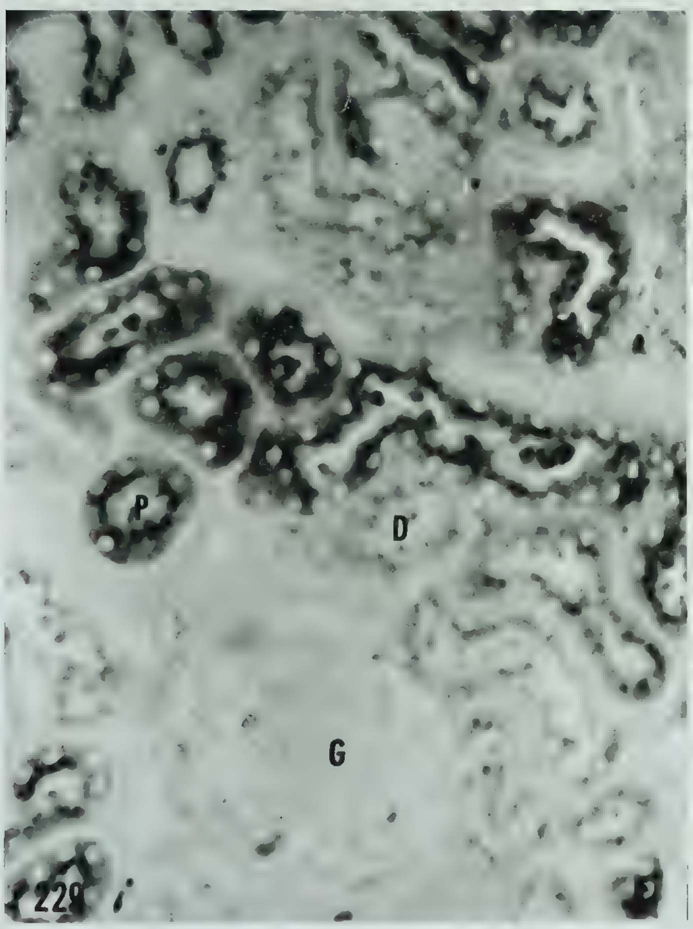

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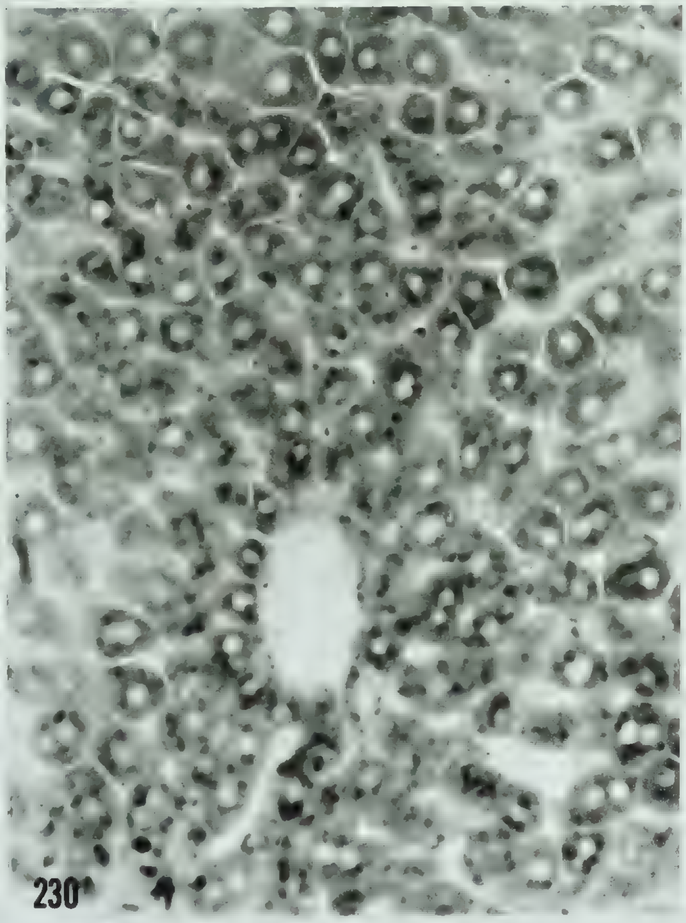
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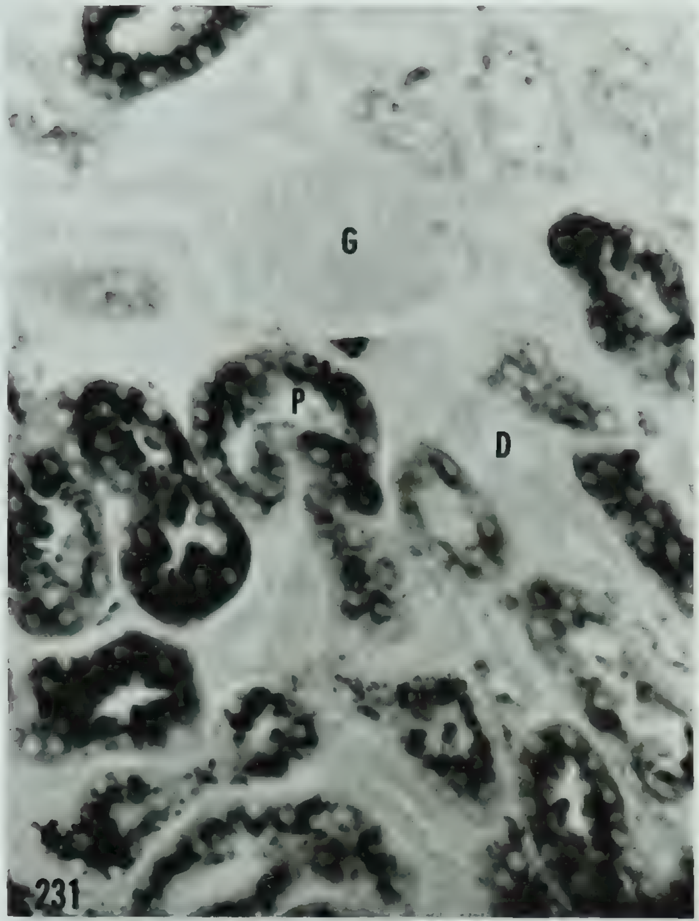
Fig. 231. Estrone experiment, (subcutaneous injection of 0.05 mg. estrone dissolved in 0.25 ml. olive oil), mouse kidney, B-glucuronidase. 250 X.

Fig. 232. Estrone experiment, (subcutaneous injection of 0.05 mg. estrone dissolved in 0.25 ml. olive oil), mouse liver, B-glucuronidase. 250 X.

Fig. 233. Normal rat kidney, control for AAF experiment, B-glycerophosphate phosphatase. 250 X.

Fig. 234. Normal rat kidney, control for AAF experiment, Naphthol AS-TR phosphate phosphatase. 250 X.



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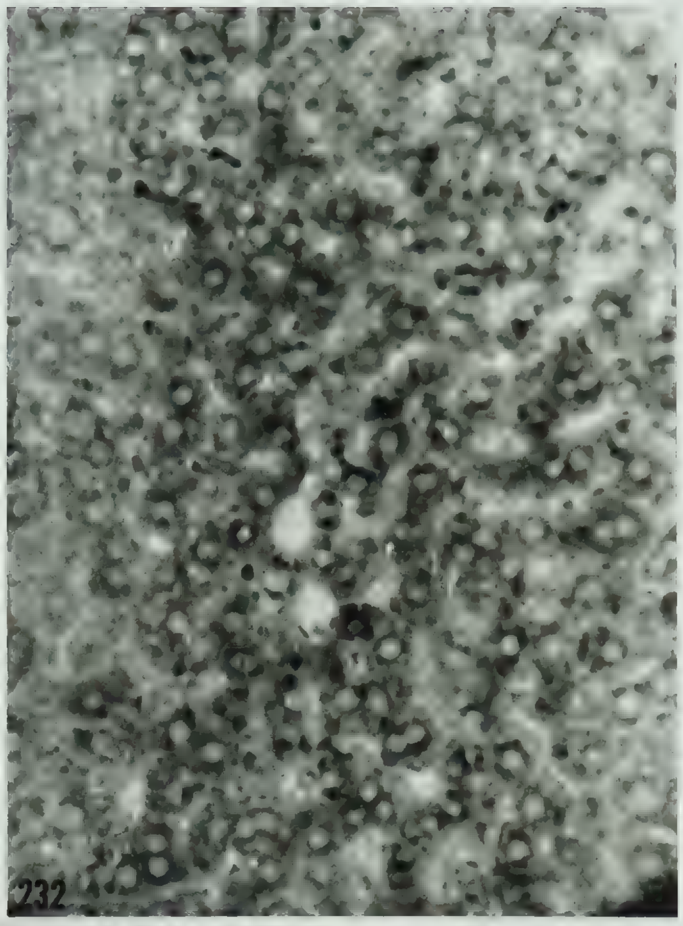

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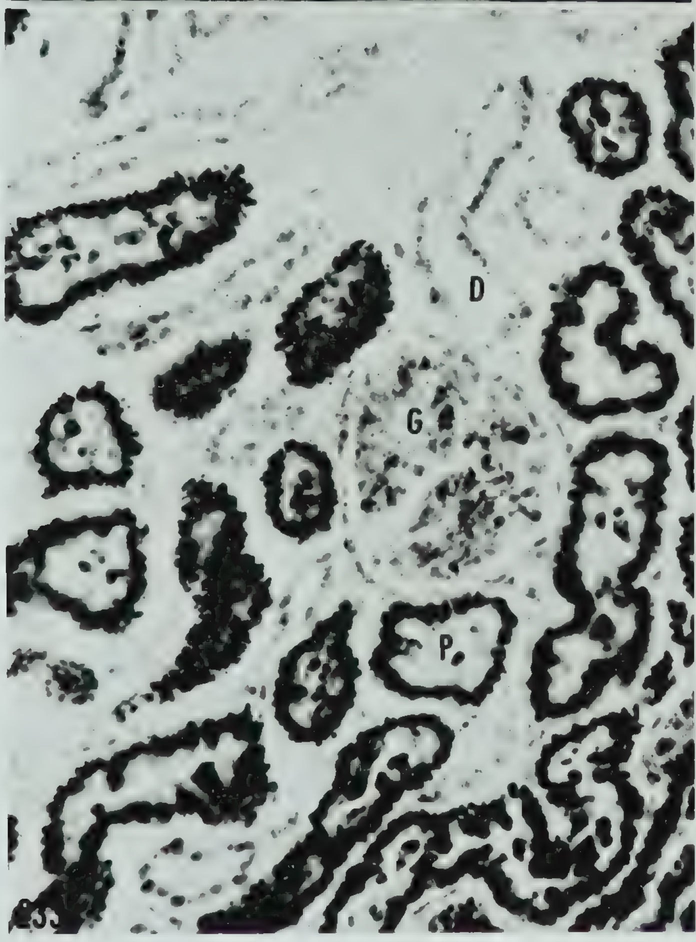

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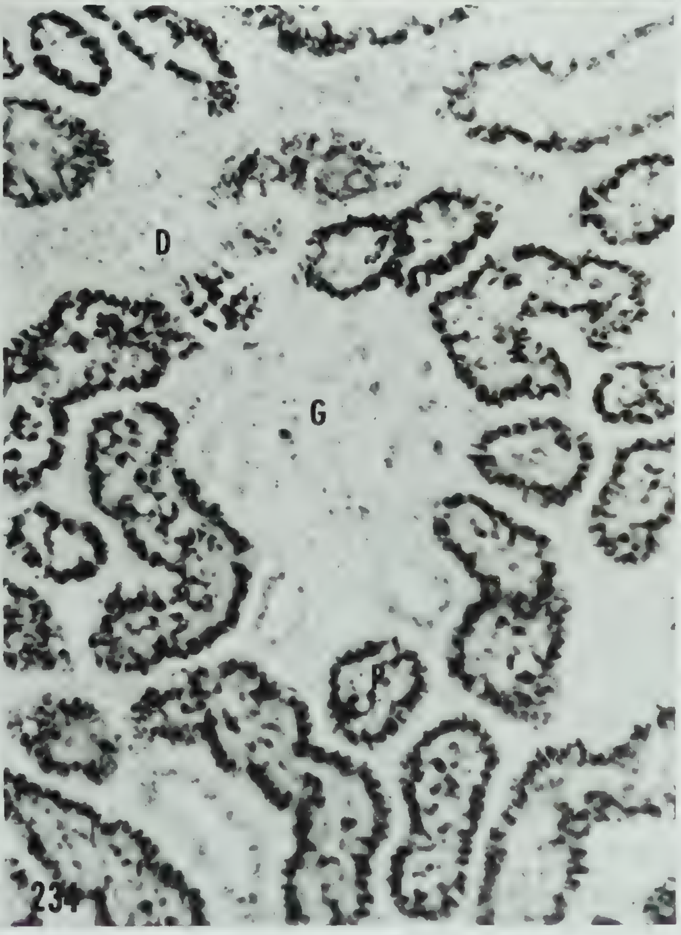

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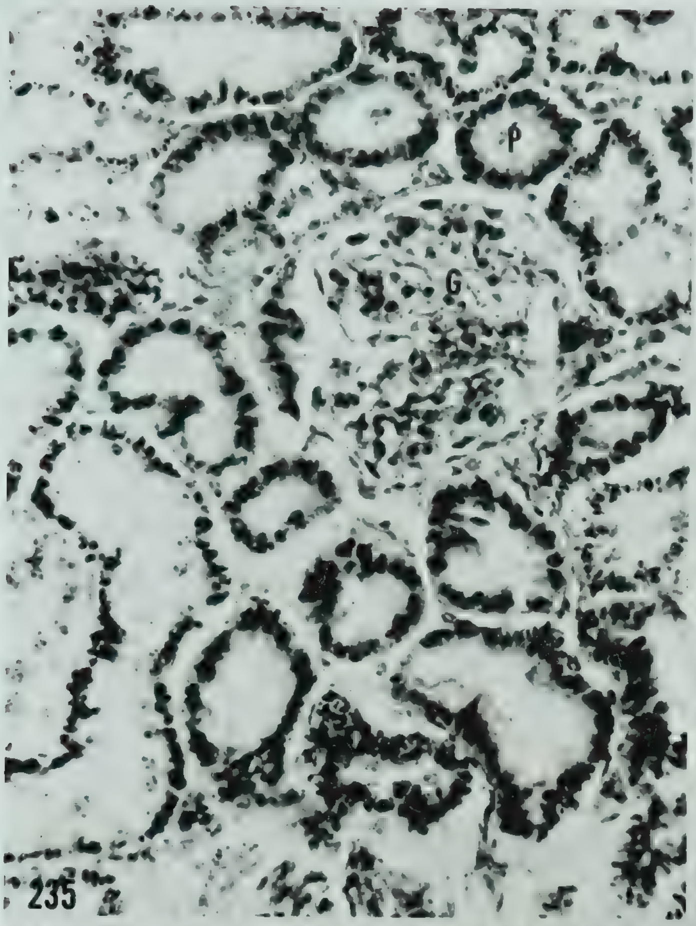
Fig. 235. AAF experiment, (oral administration of 50 mg. N-2-Fluorenylacetamide per 100 g. body weight), rat kidney, B-glycerophosphate phosphatase. 250 X.

Fig. 236. AAF experiment, (oral administration of 50 mg. N-2-Fluorenylacetamide per 100 g. body weight), rat kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 237. Normal rat liver, control for AAF experiment, B-glycerophosphate phosphatase. 250 X.

Fig. 238. Normal rat liver, control for AAF experiment, Naphthol AS-TR phosphate phosphatase. 250 X.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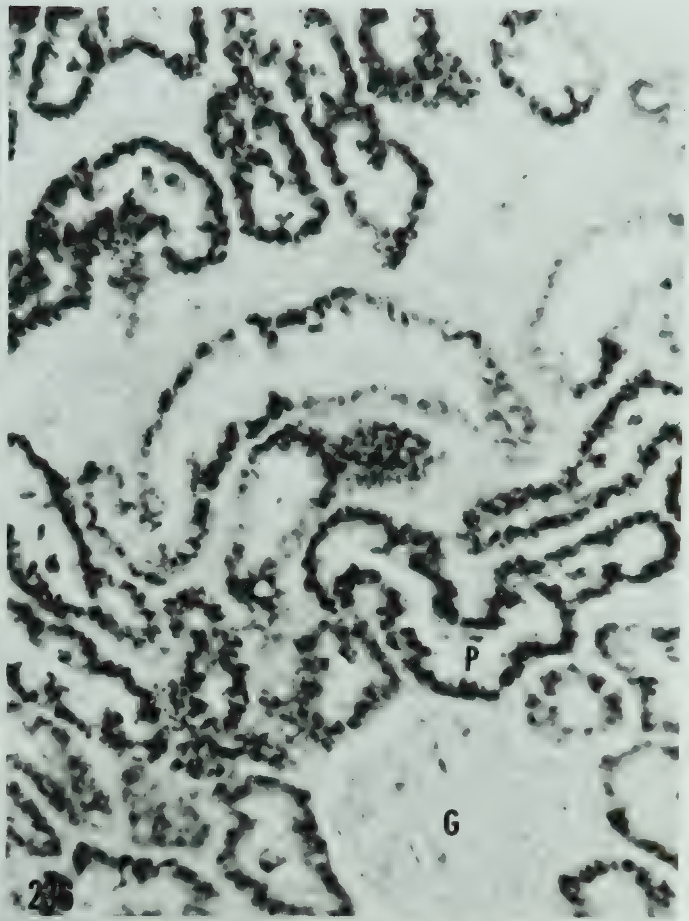
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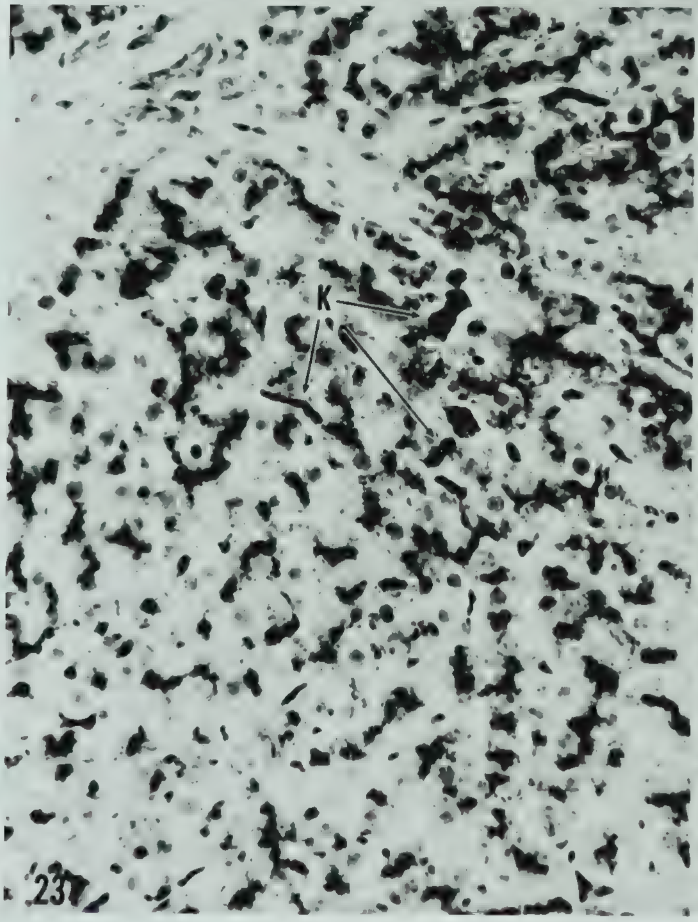
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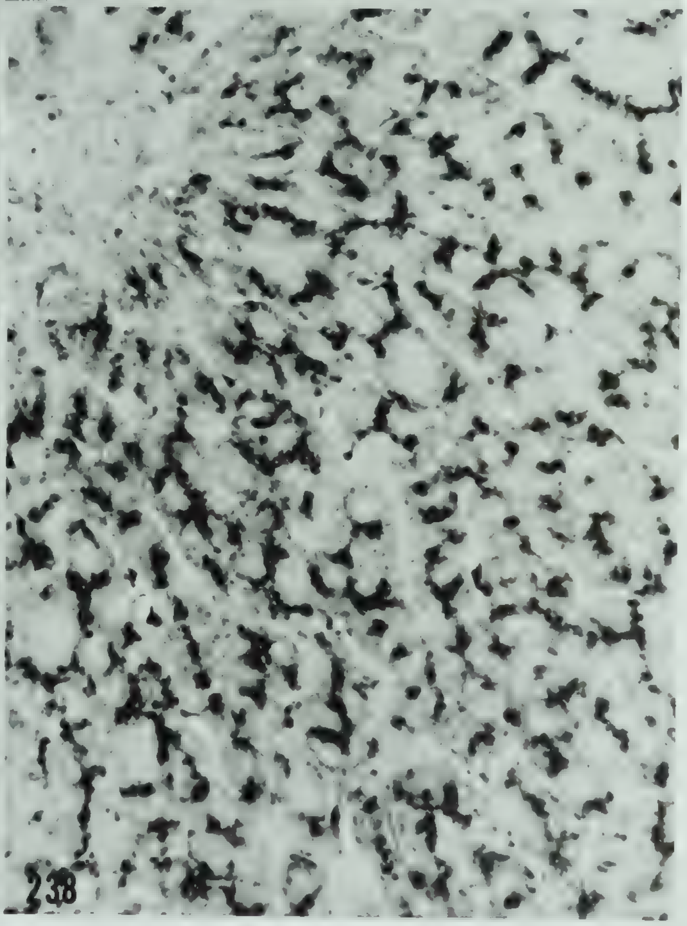
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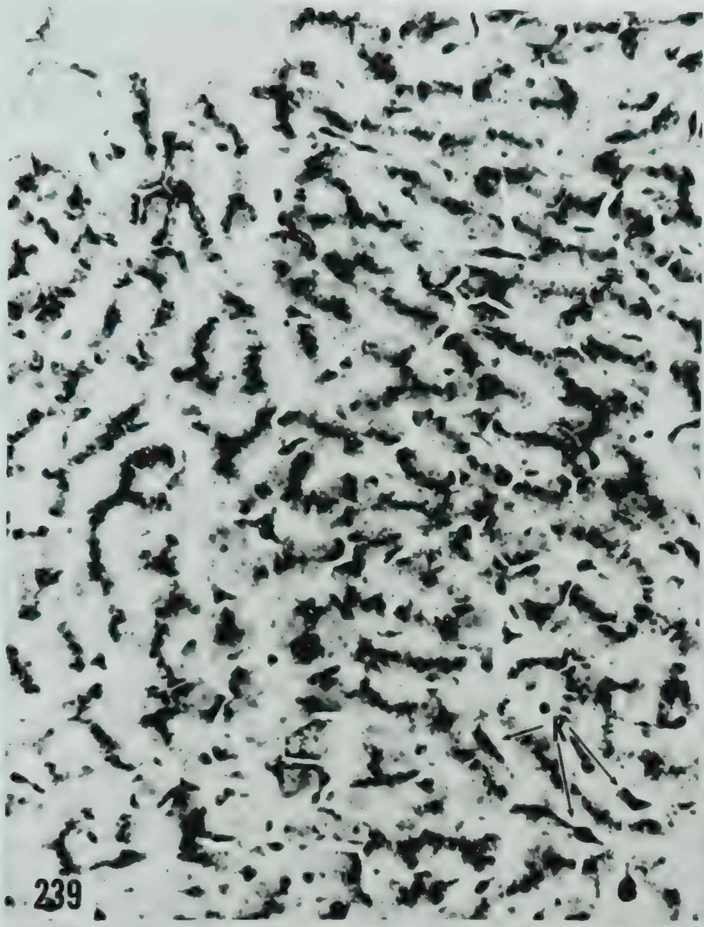
Fig. 239. AAF experiment, (oral administration of 50 mg. N-2-Fluorenylacetamide per 100 g. body weight), rat liver, B-glycerophosphate phosphatase. 250 X.

Fig. 240. AAF experiment, (oral administration of 50 mg. N-2-Fluorenylacetamide per 100 g. body weight), rat liver, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 241. Normal mouse kidney, control for dextran experiment, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 242. Normal mouse liver, control for dextran experiment, Naphthol AS-TR phosphate phosphatase. 250 X.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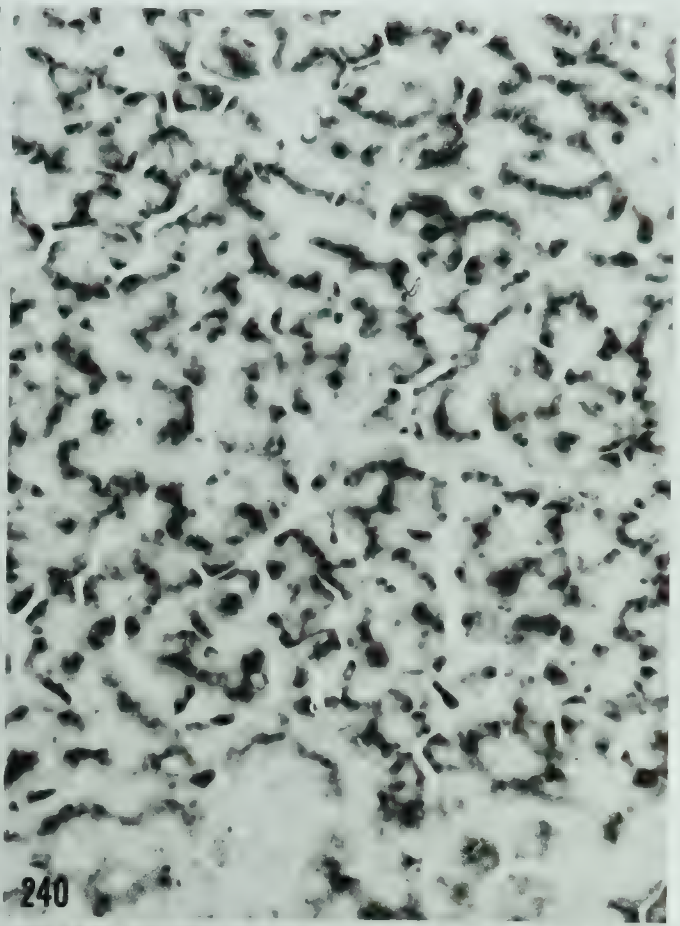
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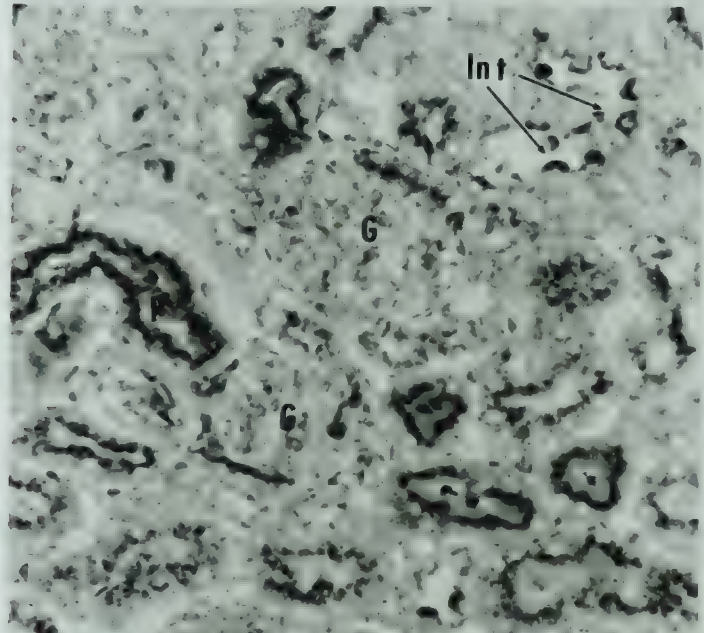
Int
G
P
G
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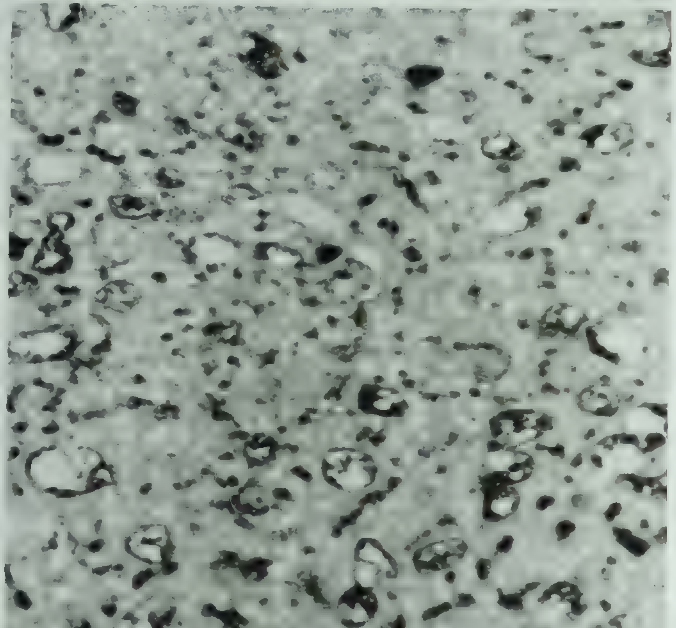
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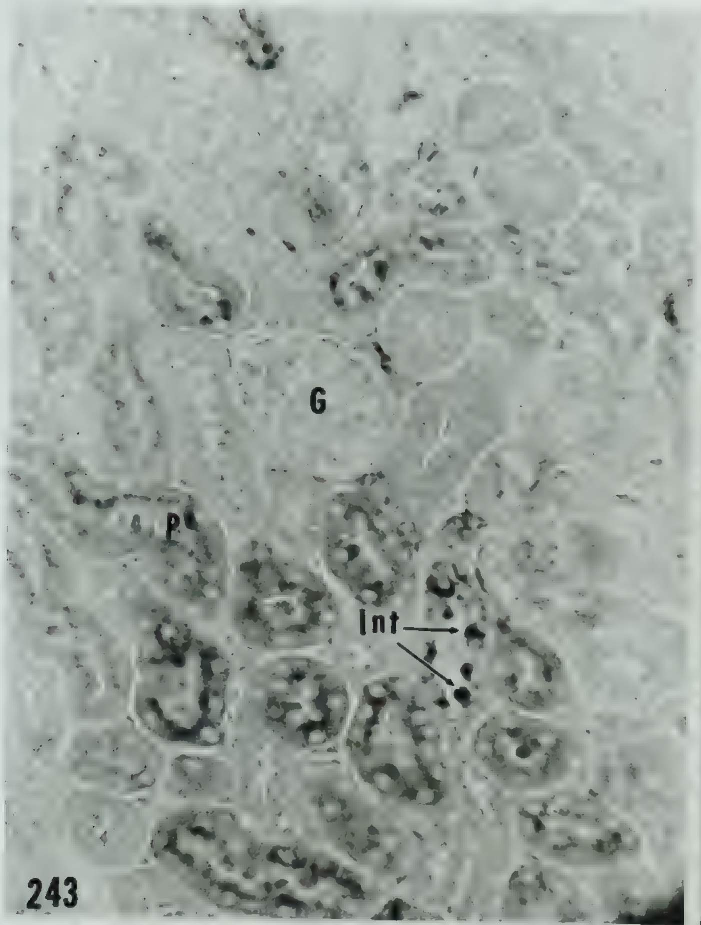
Fig. 243. Normal mouse kidney, control for dextran experiment, B-glucuronidase. 250 X.

Fig. 244. Normal mouse liver, control for dextran experiment, B-glucuronidase. 250 X.

Fig. 245. Dextran experiment,(intraperitoneal injections of 50 mg. dextran (160,000 MW) dissolved in 1 ml. normal saline on nine successive days), mouse kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 246. Dextran experiment, (intraperitoneal injections of 50 mg. dextran (160,000 MW) dissolved in 1 ml. normal saline on nine successive days), mouse liver, Naphthol AS-TR phosphate phosphatase. 250 X.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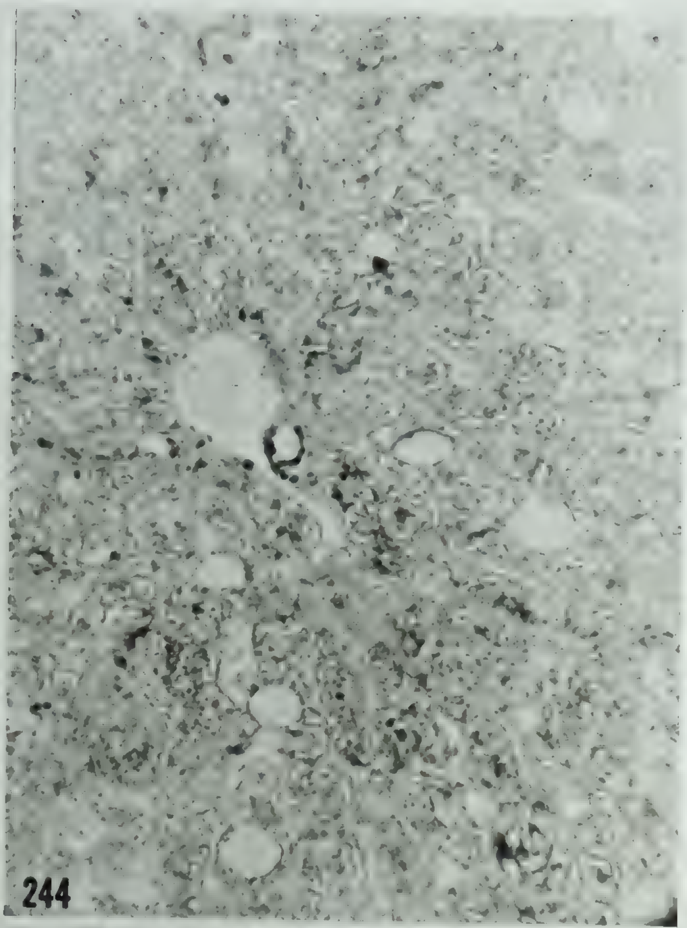
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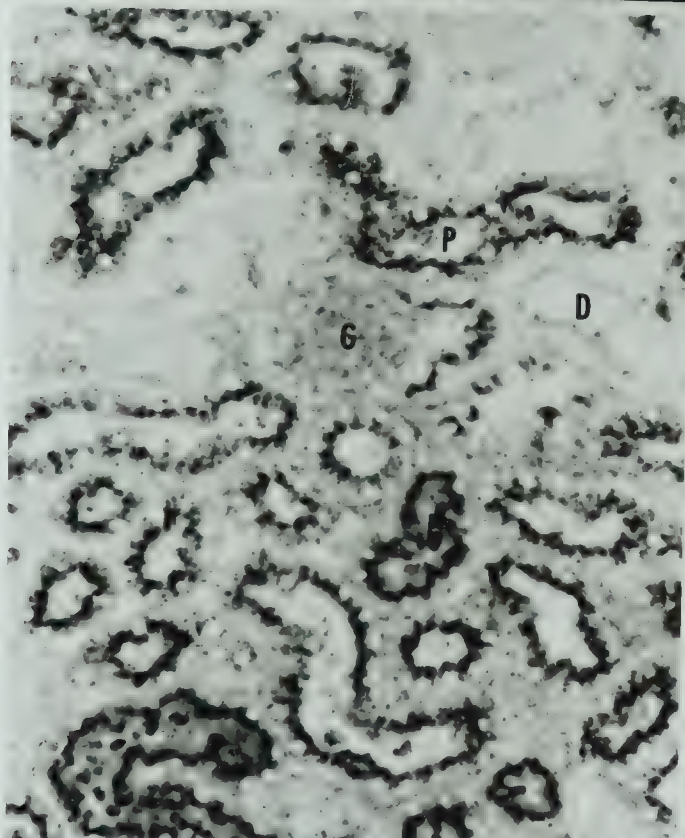
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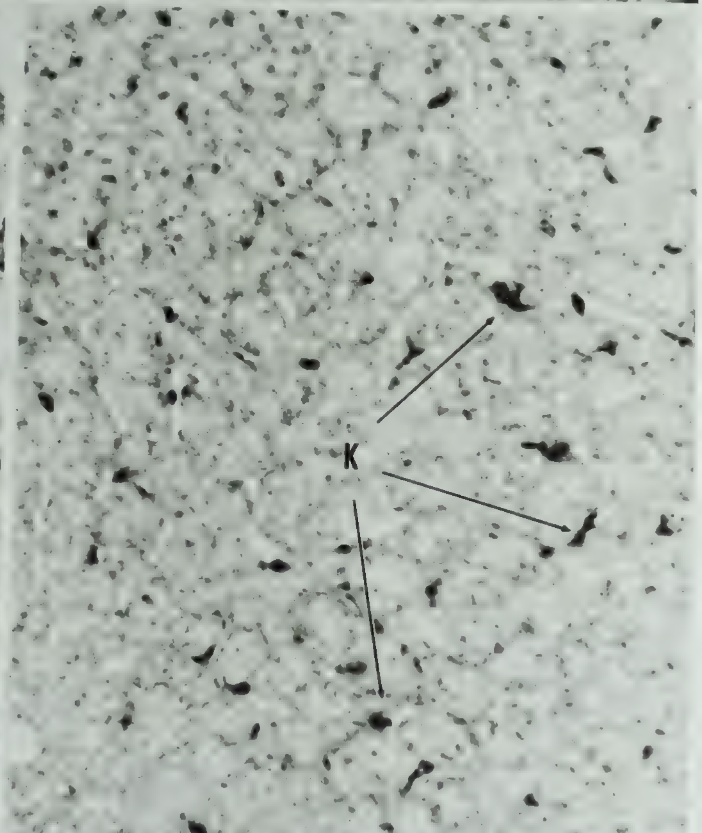
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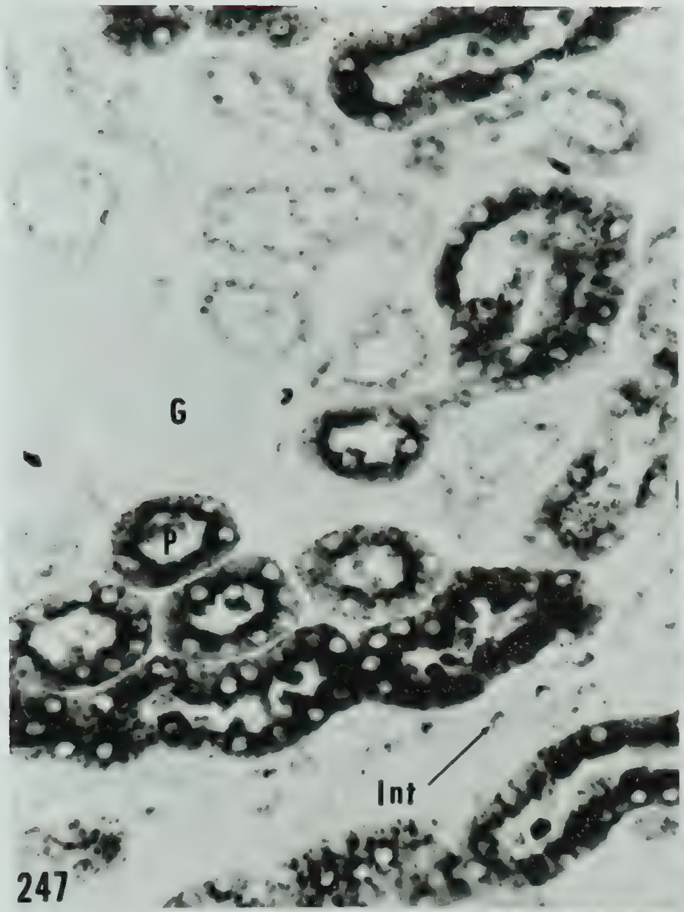
Fig. 247. Dextran experiment, (intraperitoneal injections of 50 mg. dextran (160,000 MW) dissolved in 1 ml. normal saline on nine successive days), mouse kidney, B-glucuronidase. 250 X.

Fig. 248. Dextran experiment,(intraperitoneal injections of 50 mg. dextran (160,000 MW) dissolved in 1 ml. normal saline on nine successive days), mouse liver, B-glucuronidase. 250 X.

Fig. 249. Dextran experiment, (intraperitoneal injections of 50 mg. dextran (160,000 MW) dissolved in 1 ml. normal saline on nine successive days), mouse kidney, Naphthol AS-TR phosphate phosphatase. 250 X.

Fig. 250. Dextran experiment, (intraperitoneal injections of 50 mg. dextran (160,000 MW) dissolved in 1 ml. normal saline on nine successive days), mouse liver, Naphthol AS-TR phosphate phosphatase. 250 X.



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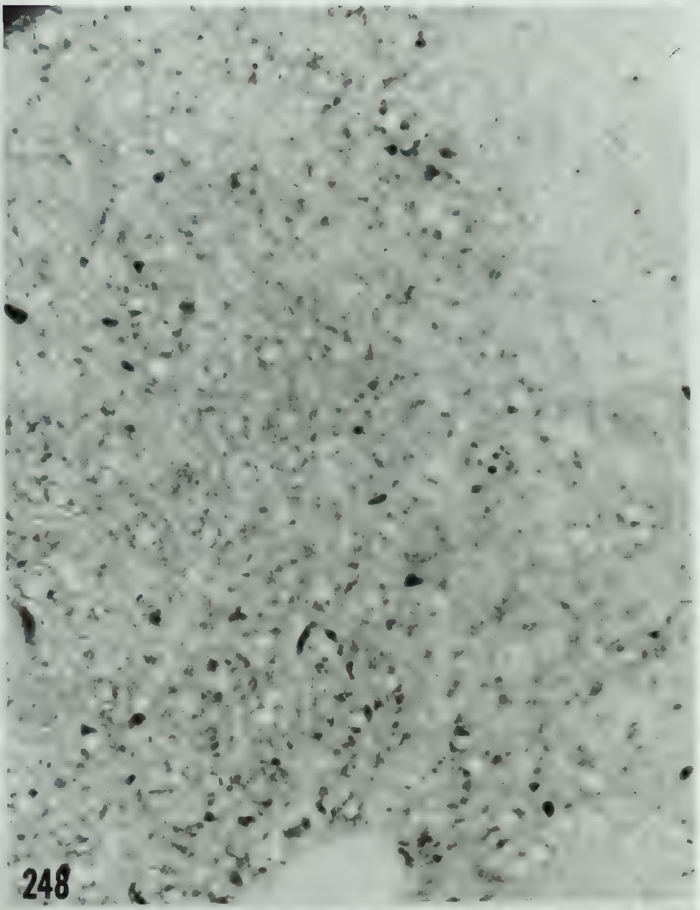

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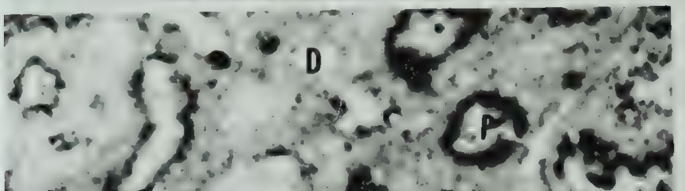

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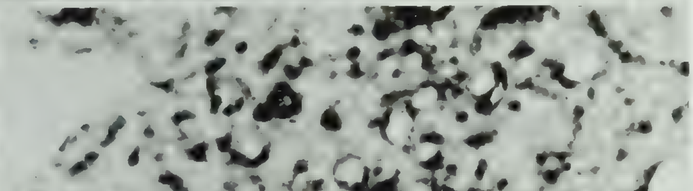

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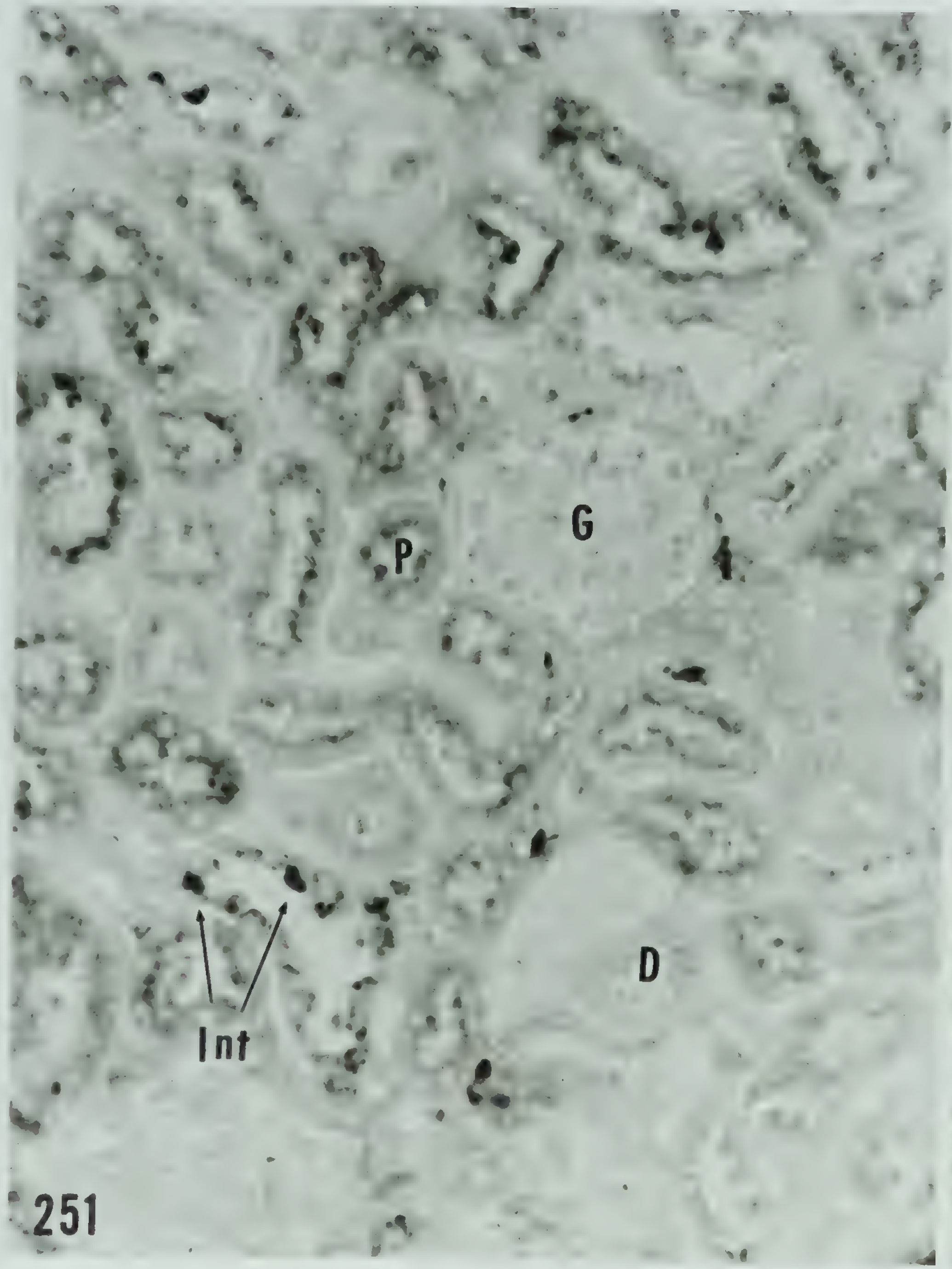
Fig. 251. Dextran experiment, (intraperitoneal injections of 50 mg. dextran (160,000 MW) dissolved in 1 ml. normal saline on nine successive days), mouse kidney, B-glucuronidase. 360 X.

Fig. 252. Dextran experiment, (intraperitoneal injections of 50 mg. dextran (160,000 MW) dissolved in 1 ml. normal saline on nine successive days), mouse liver, B-glucuronidase. 360 X.



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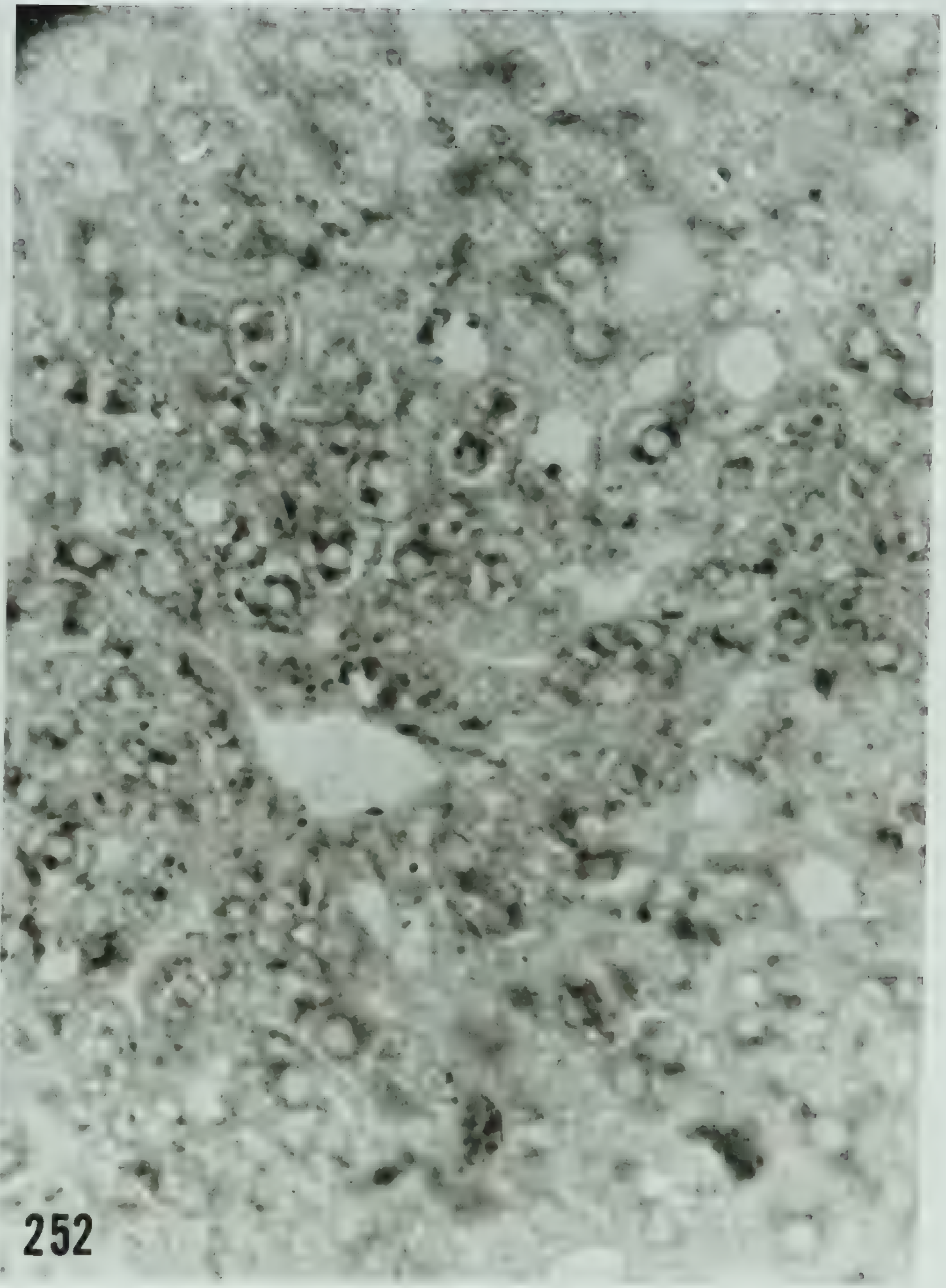

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Illustrative Electron Micrographs of Varieties of Lysosomes.

Fig. A. to Fig. L. inclusive.

LIST OF ABBREVIATIONS AND SYMBOLS

Au	Colloidal gold
Bm	Basement membrane
Ca	Colloidal carbon
Ce	Centriole
Go	Golgi apparatus
L	Lumen
Ly	Lysosome
Mb	Microbody
Mi	Mitochondrion
Nu	Nucleus
Pi	Pinocytotic vacuole
Rb	Residual body
Si	Sinusoidal space

Fig. A. Electron micrograph of metamyelocyte in erythropoietic focus in foetal rat liver. Membrane-bound cytoplasmic granules (Ly) are primary lysosomes. 10,000 X.

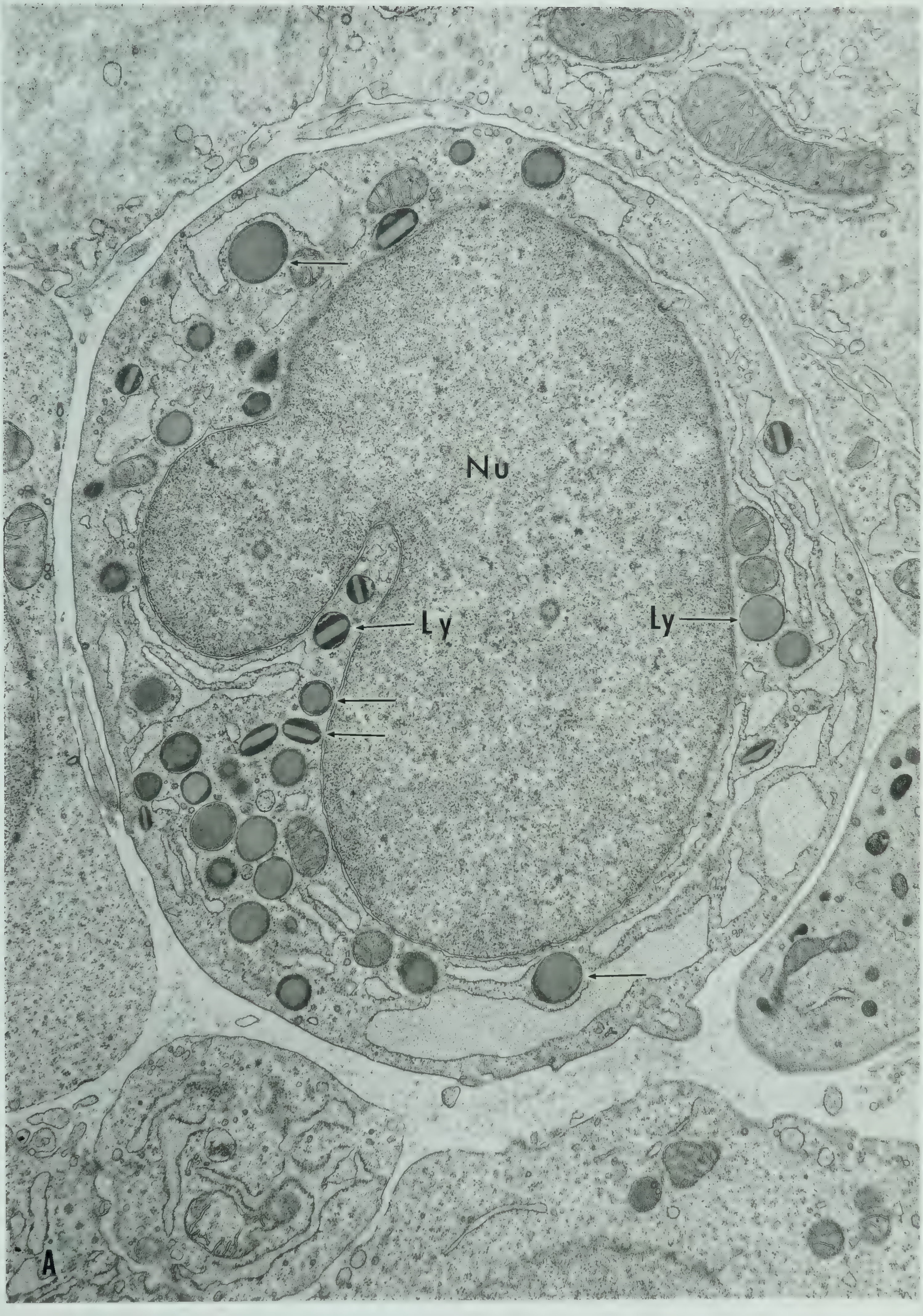

Nu
Ly
Ly
A

Fig. B. Ultrastructural appearances of phagolysosomes (Ly) in epithelium lining proximal tubule of rat kidney. 30,000 X.

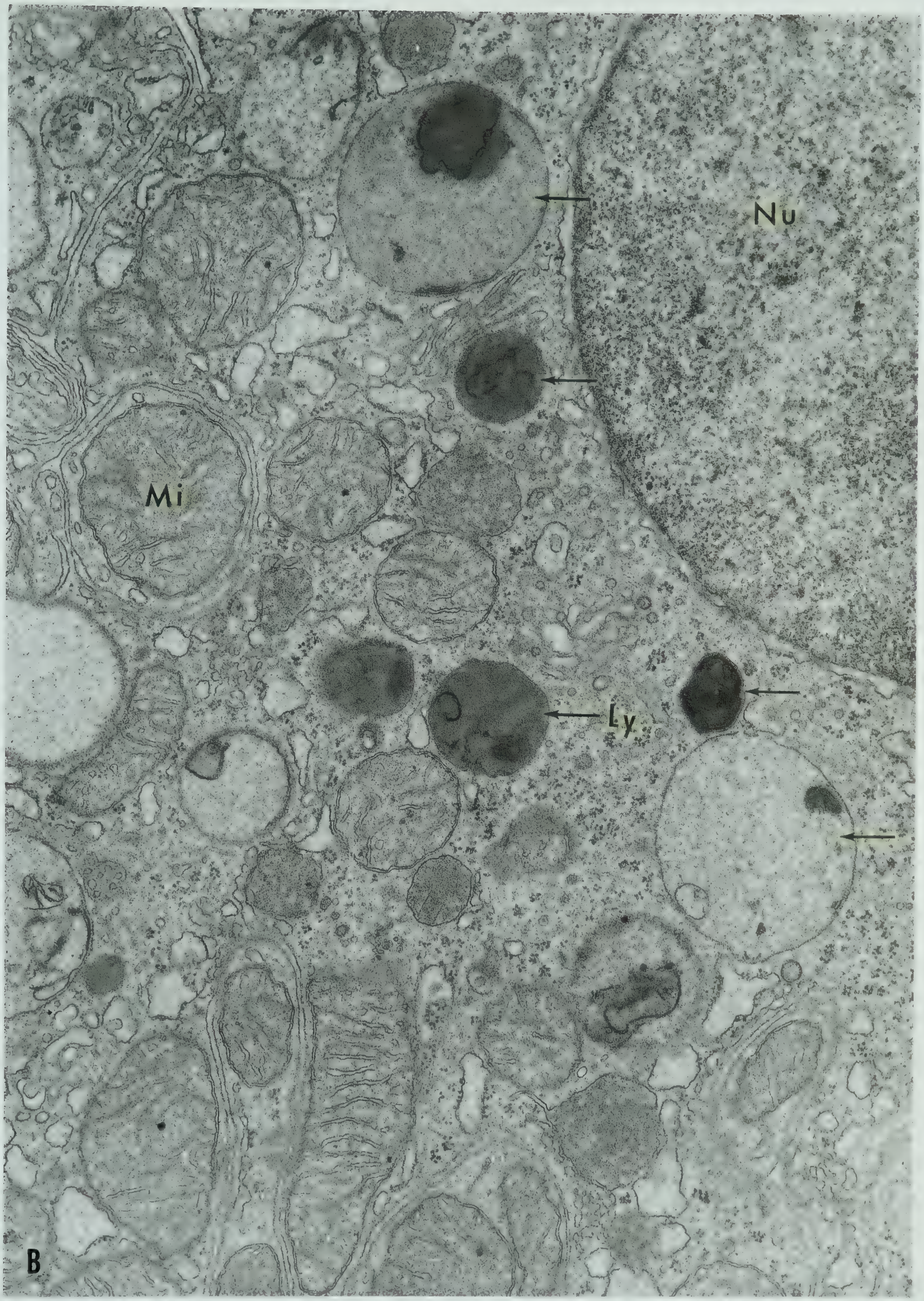

Nu
Mi
Ly
B

Fig. C. Electron microscopic cytochemical localization of acid phosphatase activity to lysosomes (Ly) in proximal tubule of rat kidney. Gomori's lead phosphate method. 30,000 X.

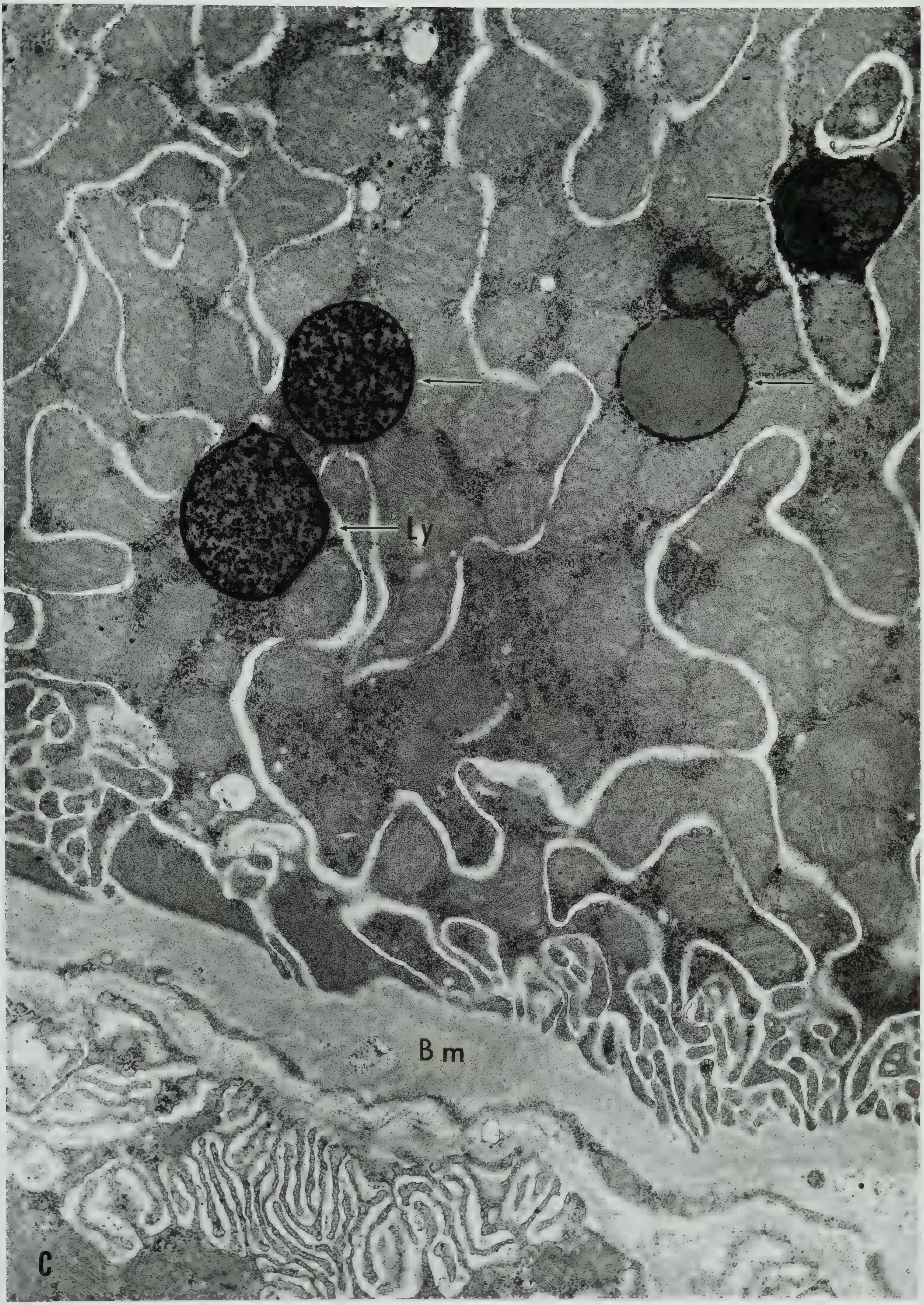

Ly
Bm
C

Fig. D. Survey electron micrograph of proximal convoluted tubule of rat kidney showing phagolysosomes (Ly) in mid portions of two cells. 10,000 X.

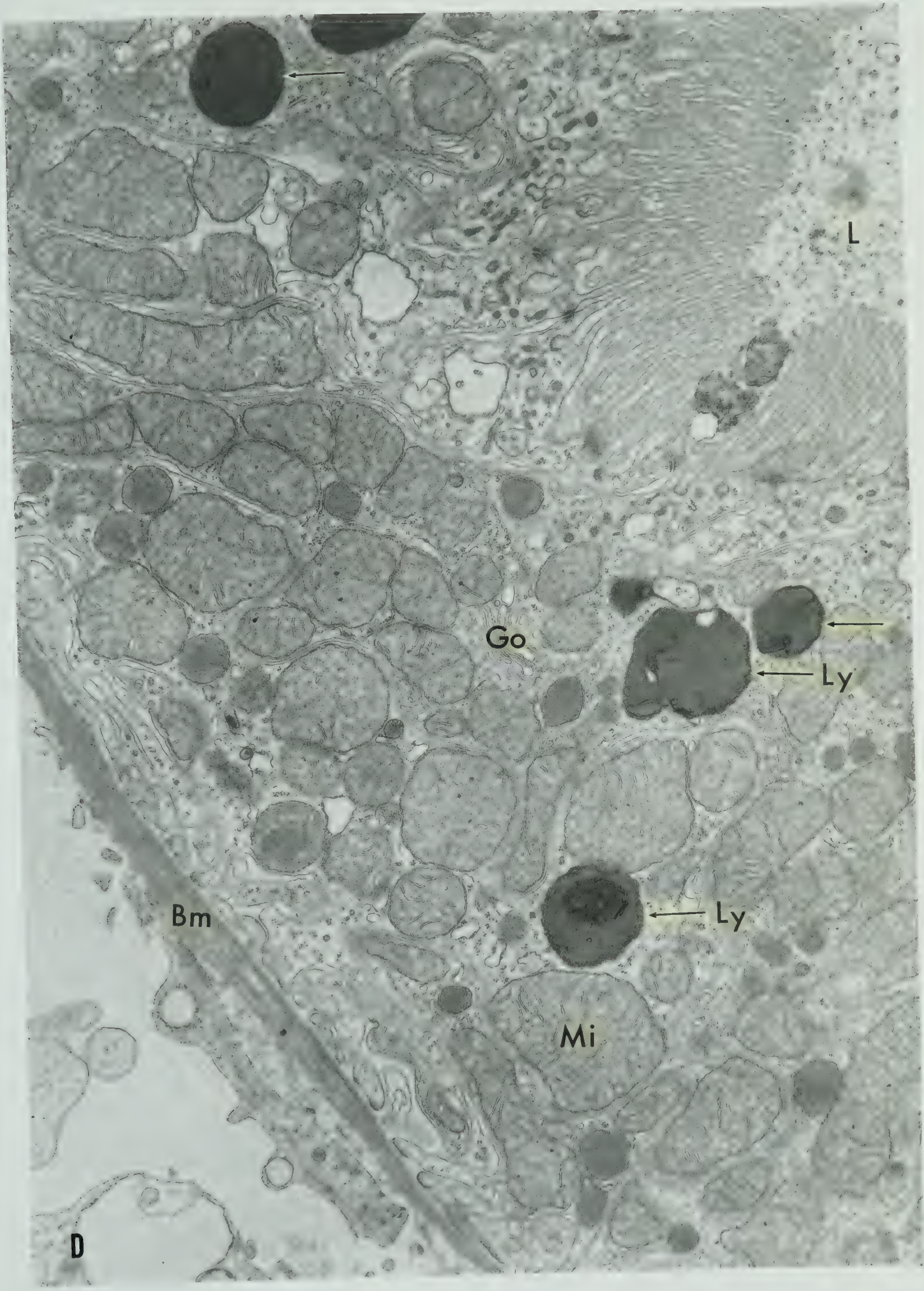

L
Go
Ly
Ly
Bm
Mi
D

Fig. E. Golgi region of rat liver cell showing a probable origin of primary lysosomes from Golgi vacuoles as well as several derived (secondary) lysosomes (Ly). 30,000 X.

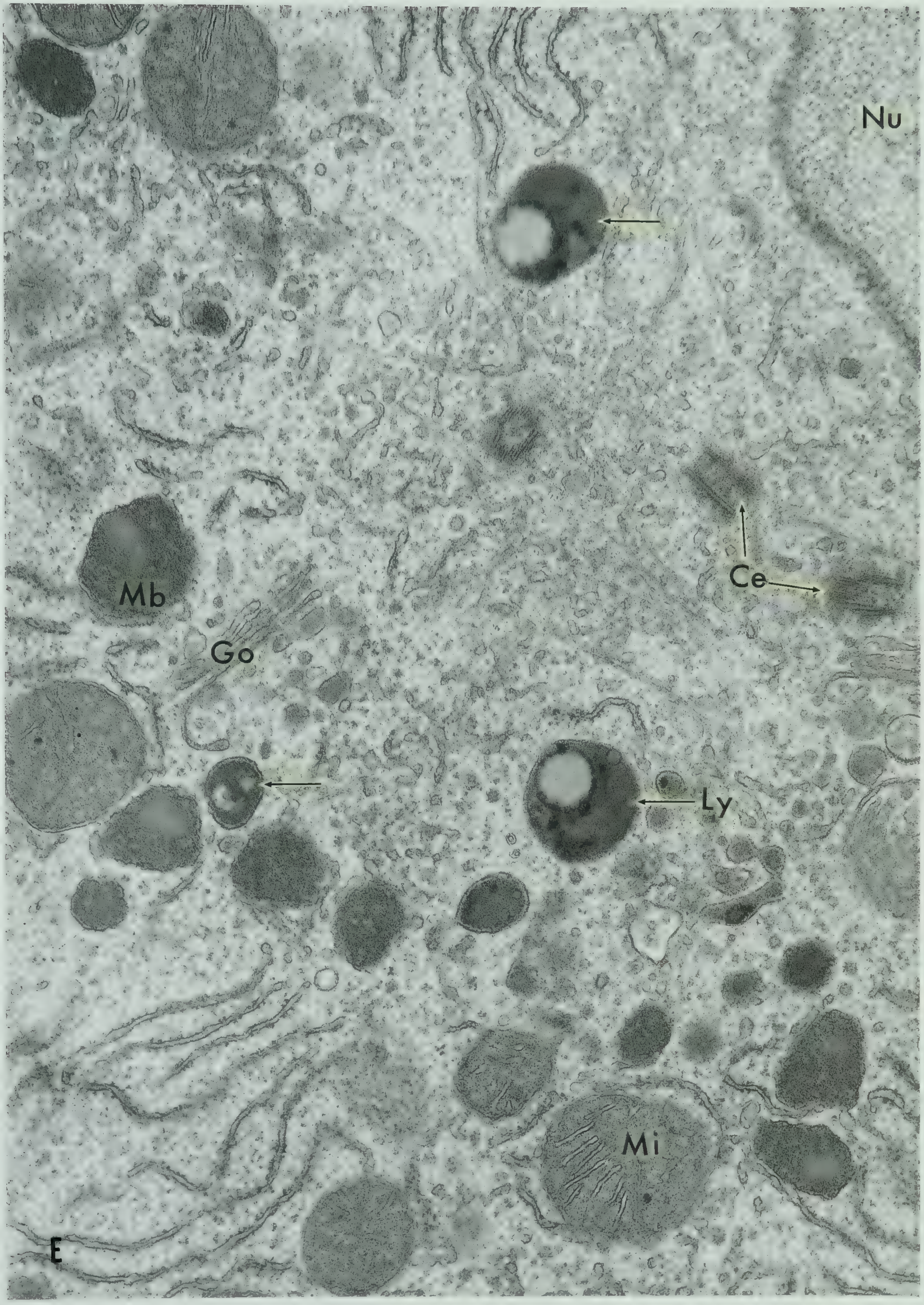

Nu
Mb
Go
Ce
Ly
Mi
E

Fig. F. Phagolysosomes in macrophage of mouse spleen. Contained materials, including ferritin, lipid, and phospholipid, give evidence of previous participation in several digestive events. 30,000 X.

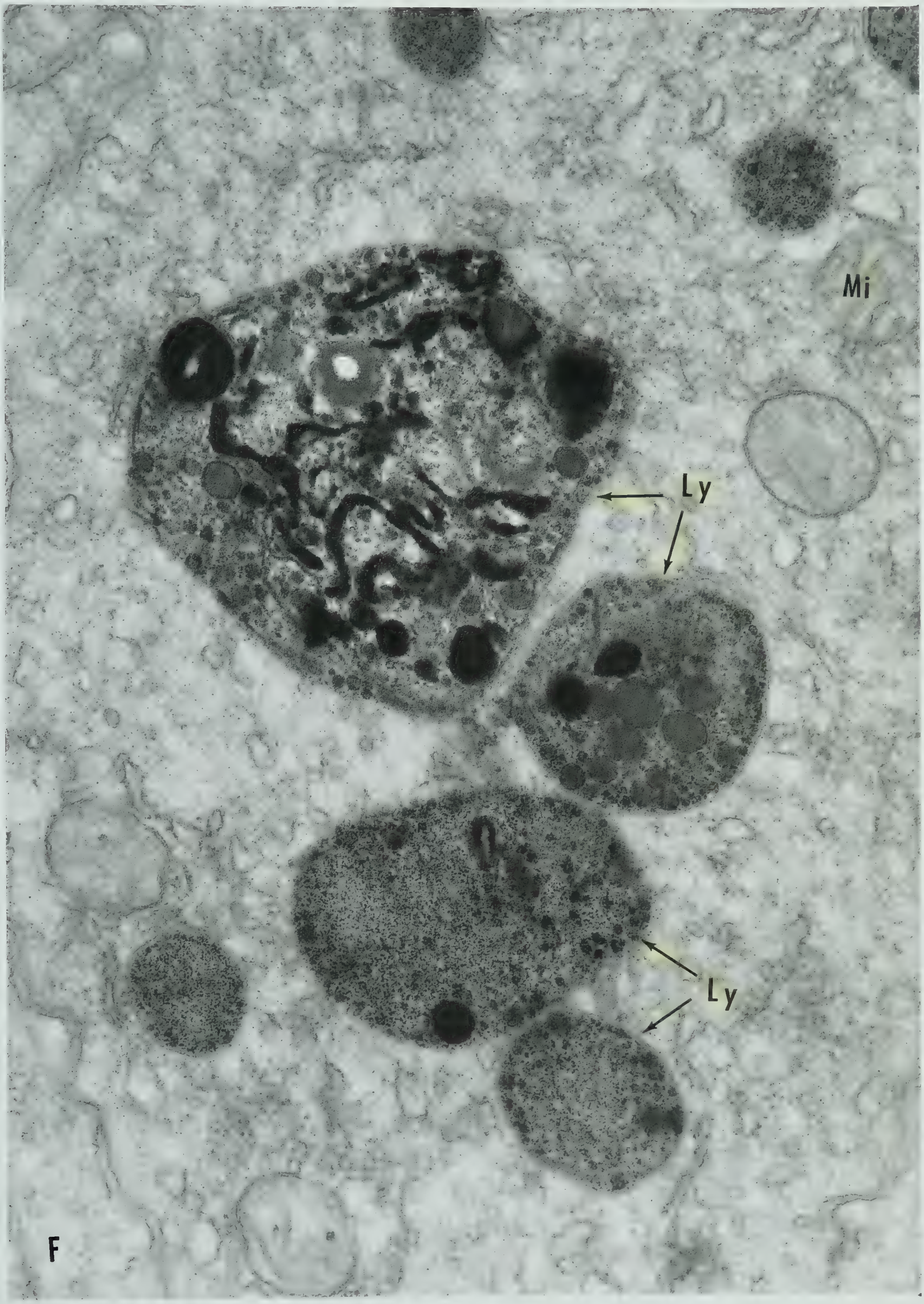

Mi
Ly
Ly
F

Fig. G. In this experiment successive pulse doses of colloidal carbon (Ca) and colloidal gold (Au) were administered intravenously at 0 and 5 hours respectively. Sacrifice was carried out 5 minutes after the last administration. Both tracer materials have entered Kupffer cells of liver by phagocytosis. Colloidal carbon has accumulated in phagolysosomes (Ly). Colloidal gold is present in pinocytotic vacuoles (Pi) principally. 20,000 X.

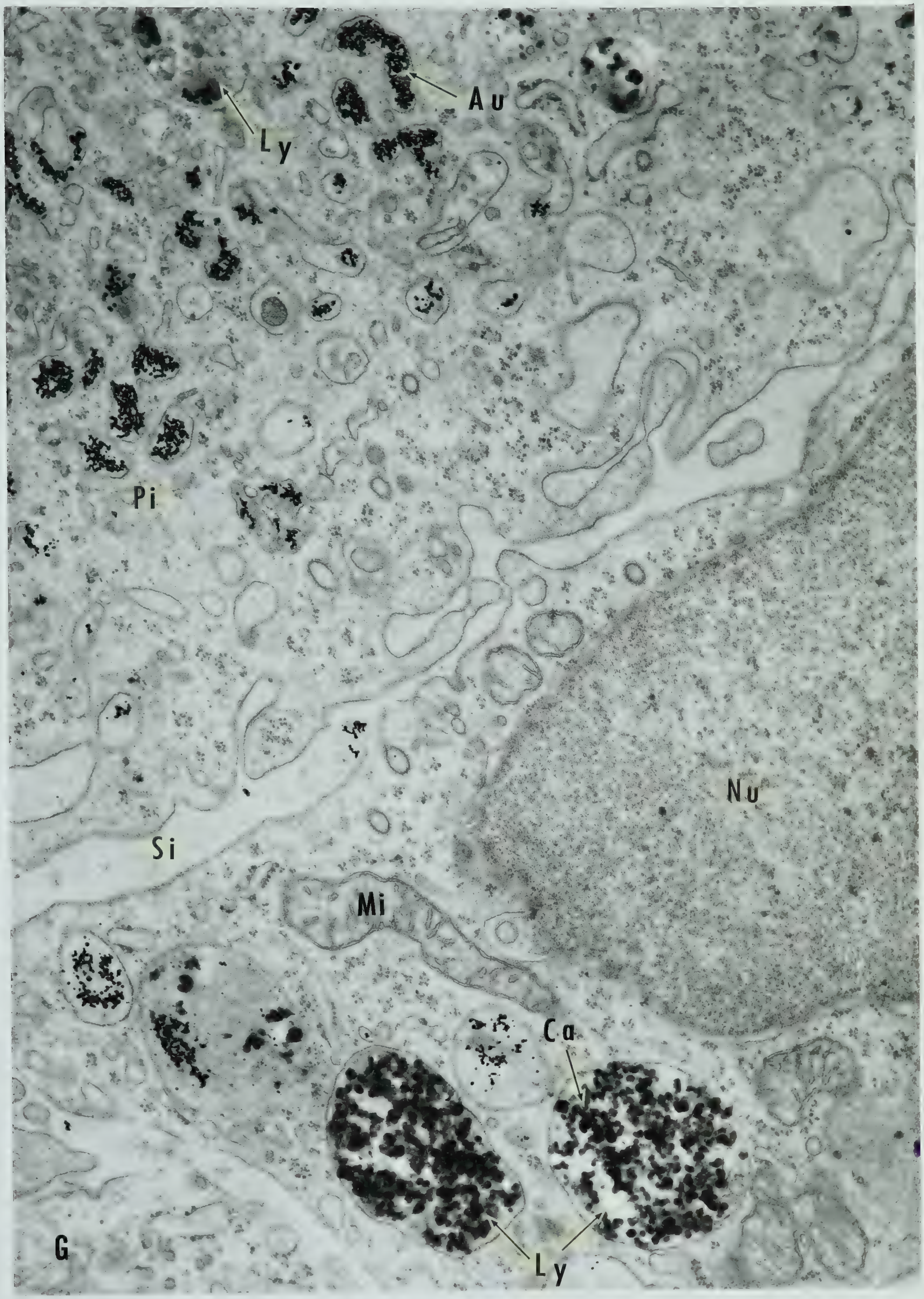

Au
Ly
Pi
Si
Nu
Mi
Ca
Ly
G

Fig. H. Experimental conditions as for Fig. G. Sacrifice, however, was performed one hour after the intravenous administration of colloidal gold (Au). Almost all of the colloidal gold has now been sequestered in phagolysosomes (Ly). 20,000 X.

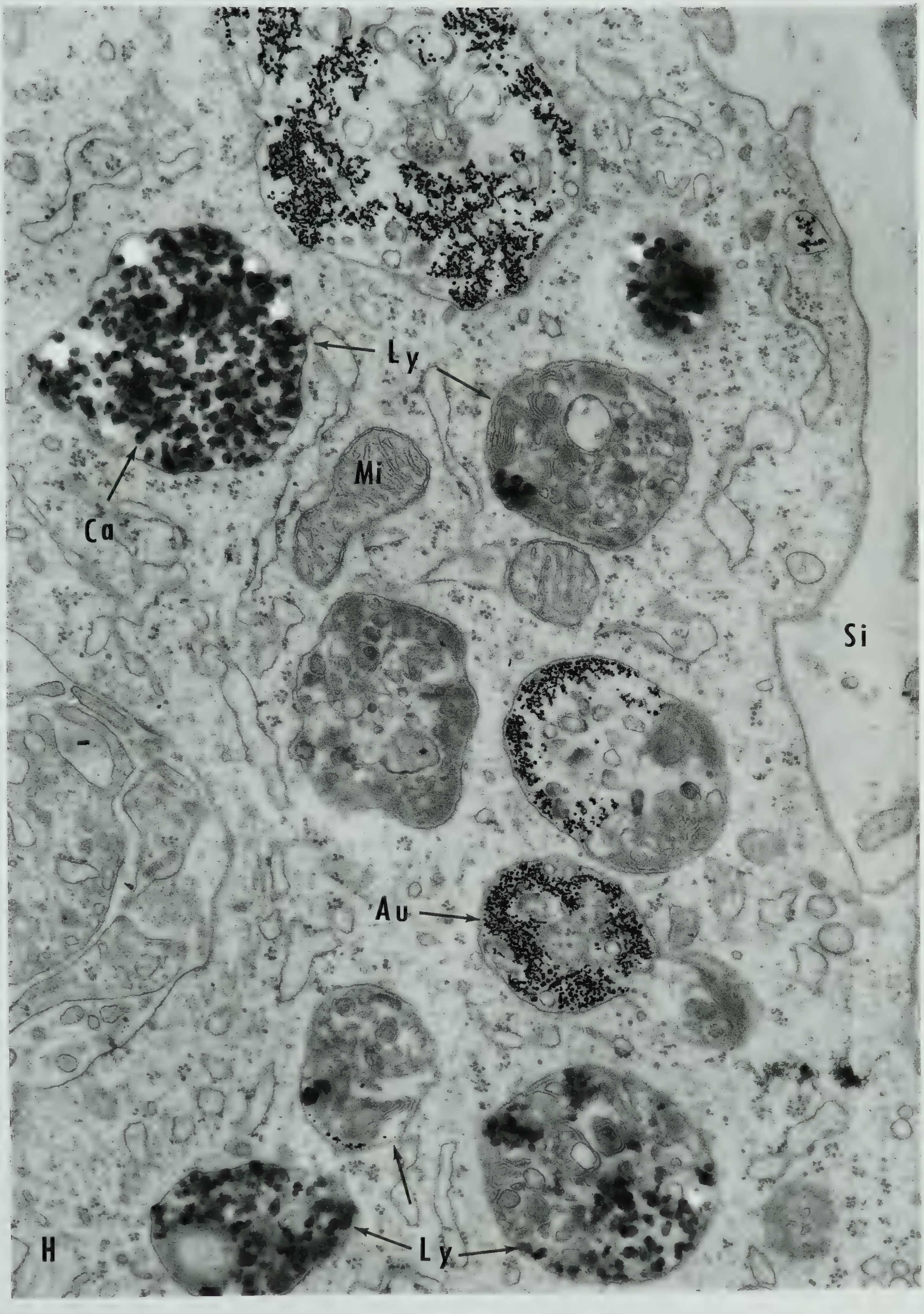

Ly
Ca
Mi
Si
Au
H
Ly

Fig. I. Cytolysosomes (Ly) induced in rat liver by the intraperitoneal administration of 100 µg. of glucagon two hours prior to sacrifice. 20,000 X.

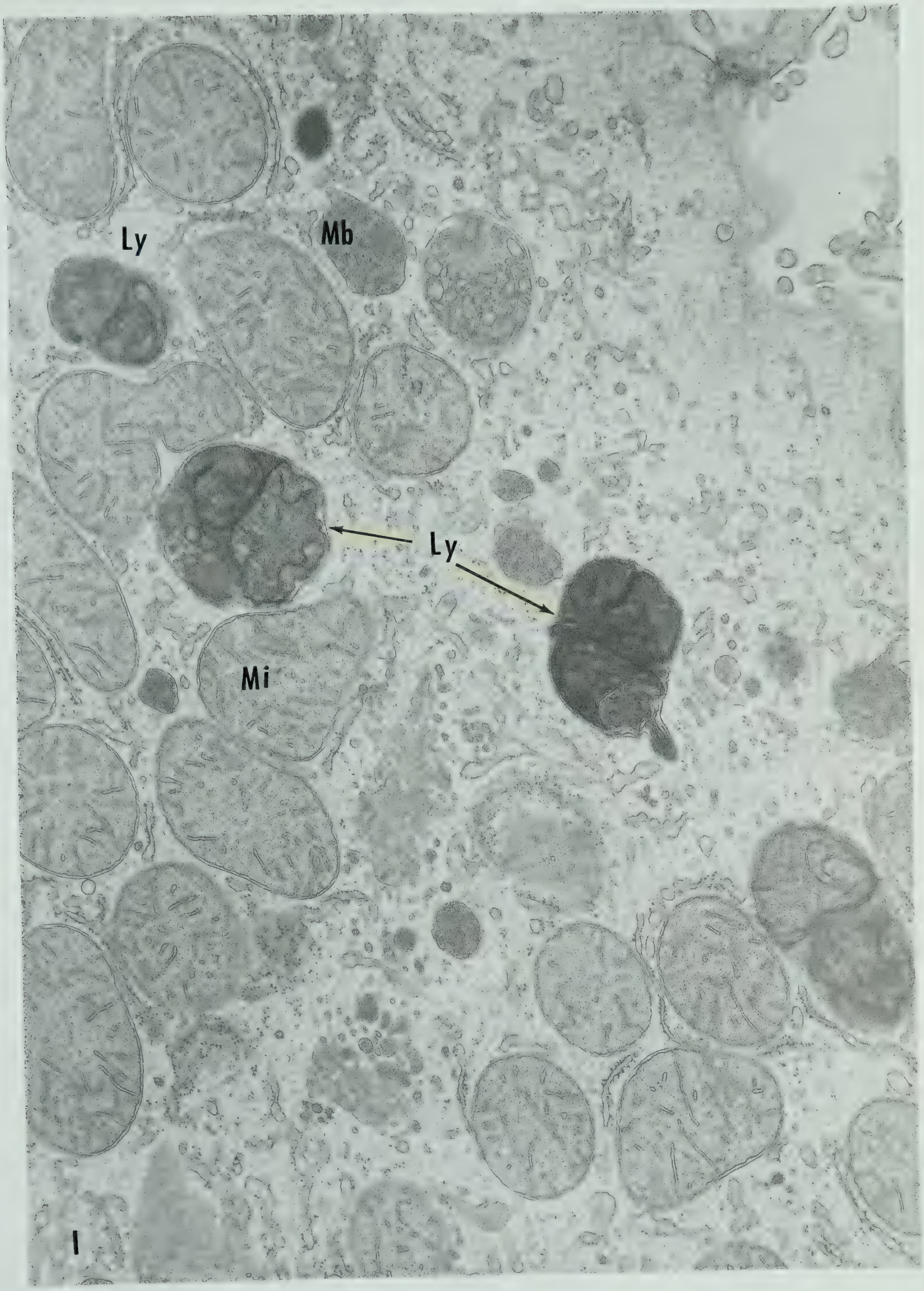

Ly
Mb
Ly
Mi
1

Fig. J. Material and experimental conditions as for Fig. I. Cytolysosomes (Ly) enclose degenerate mitochondria and portions of endoplasmic reticulum. 30,000 X.

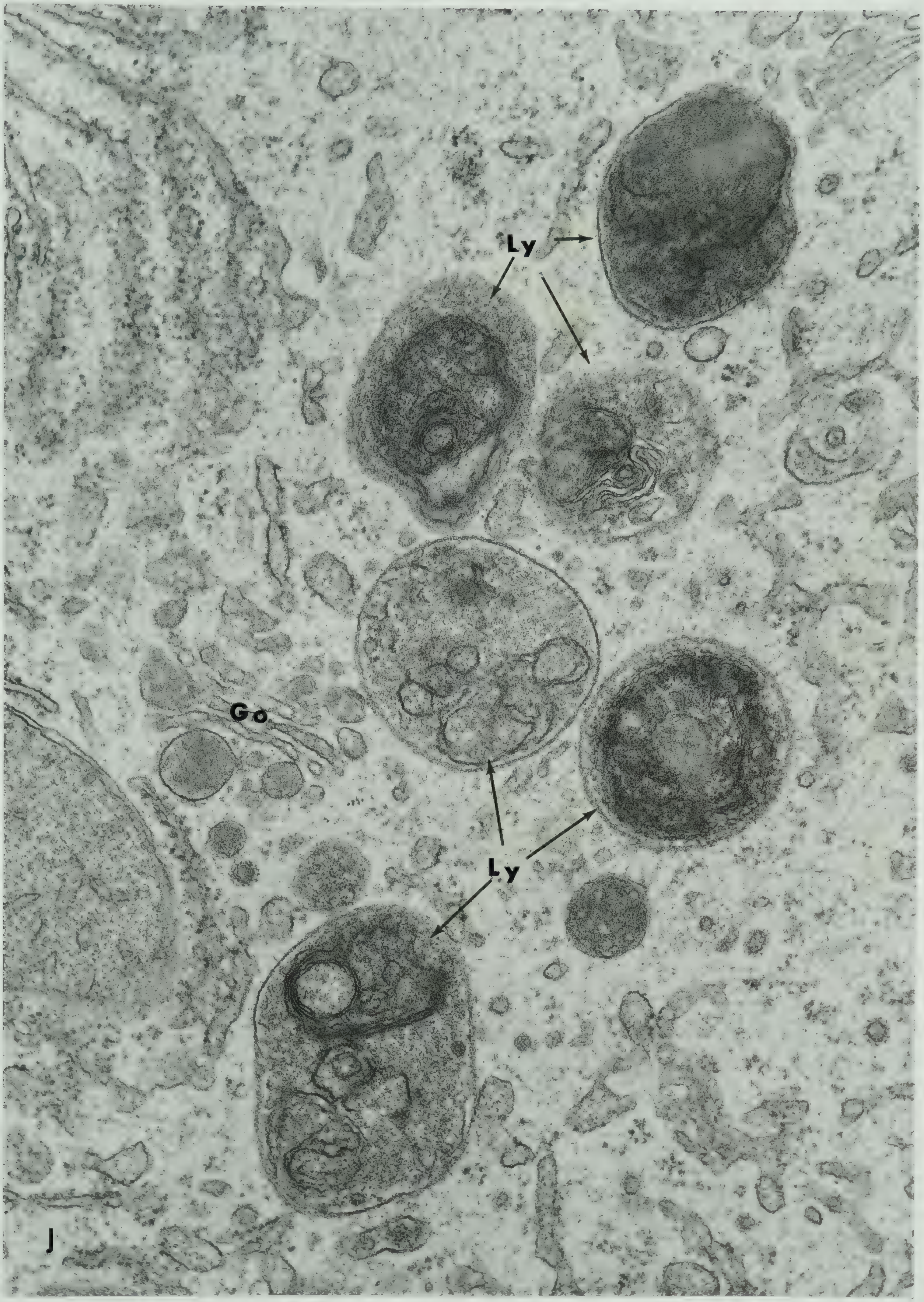

Ly
Go
Ly
J

Fig. K. Electron micrograph of incisional biopsy from human liver. Residual bodies (Rb) derived from degenerate lysosomes show transition to lipofuscin pigment. 10,000 X.

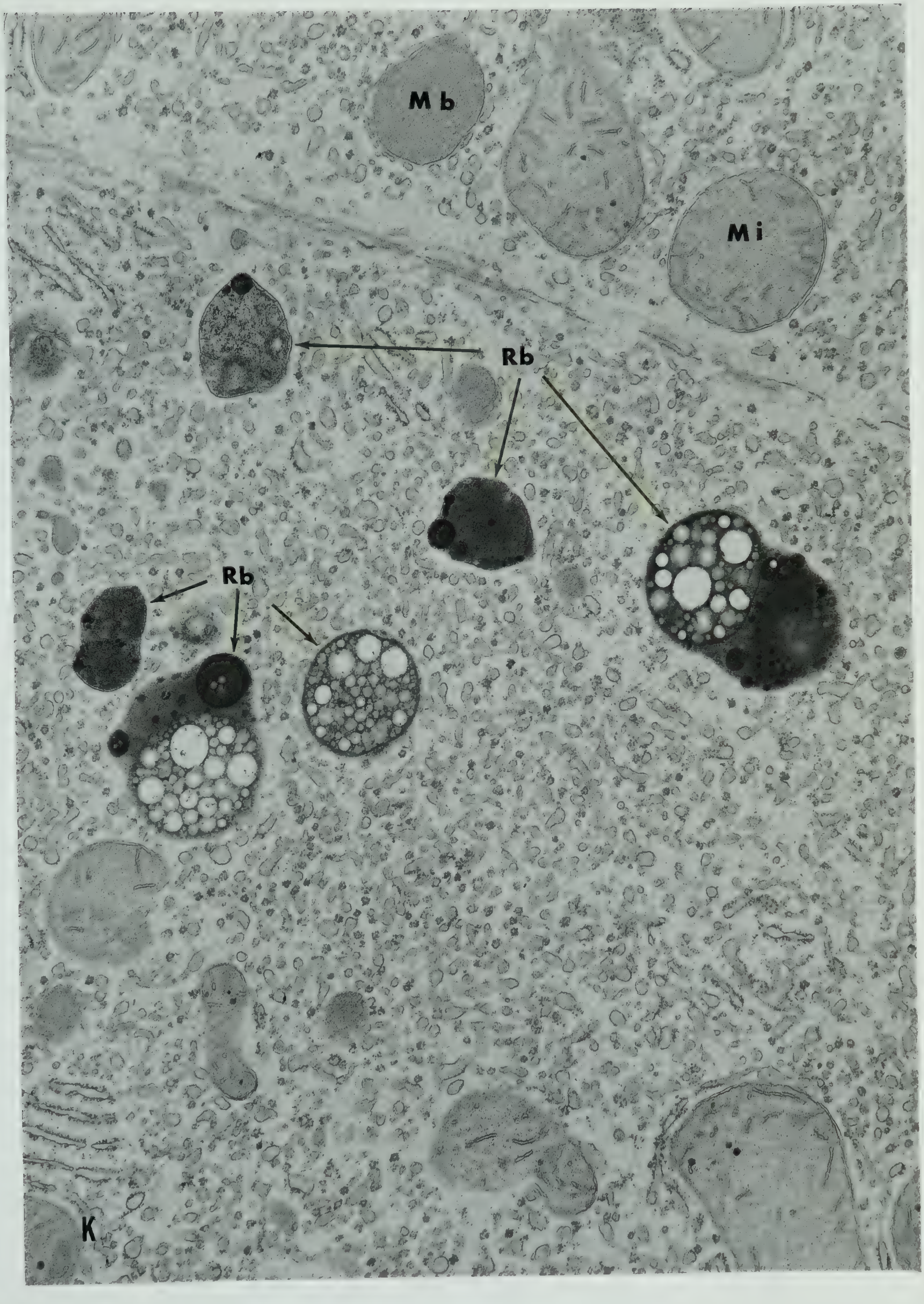
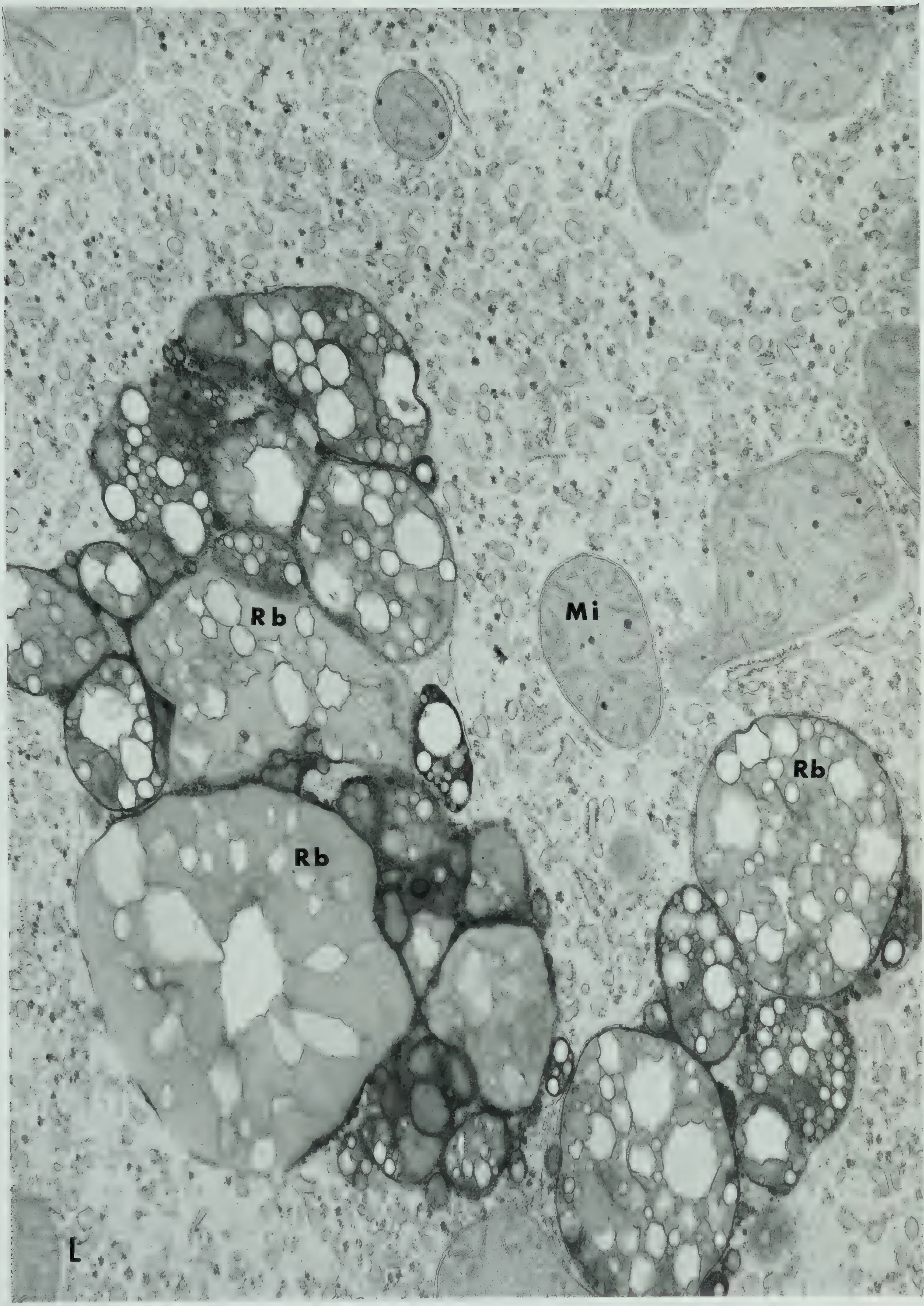

Mb
Mi
Rb
Rb
K

Fig. L. Granules of lipofuscin pigment in human liver apparently are formed by a coalescence of residual bodies (Rb) and lipid bodies. 20,000 X.


Rb
Mi
Rb
Rb
L

IV. DISCUSSION

Following the initial isolation and characterization of acid hydrolases from lysosomes of rat liver by de Duve and his collaborators in 1955, a number of reports have appeared in the literature concerning the occurrence of similar lysosomal enzymes in other tissues (de Duve, 1959, 1966; Novikoff, 1961; Verity, 1965; Weissman, 1965, 1967; Woessner, 1965; Gahan, 1967; Straus, 1967). At least a score of acid hydrolases that participate in the degradation of nucleic acid, proteins, glycogen and mucopolysaccharides have now been localized to lysosomal particles, principally of hepatic origin (de Duve, 1959, 1963, 1966; Novikoff, 1961; Weissman, 1965, 1967; Gahan, 1967; Straus, 1967). In parallel with the foregoing, a number of studies employing histochemical staining methods for acid phosphatase have provided information on the cytologic distribution of lysosomes in diverse tissues under physiologic and pathologic conditions (Novikoff, 1961; Woessner, 1965; Gahan, 1967; Straus, 1967). Suitable histochemical methods are currently available for six lysosomal acid hydrolases, but the histochemical distribution of these enzymes has not been surveyed in detail in different tissues, or in homologous tissues of different species. Until recently, the wide occurrence of acid phosphatase in animal tissues has been the basis for its use as a "marker enzyme" for lysosomes generally. In a recent histochemical study of the localization and intensity of staining of three lysosomal enzymes (acid phosphatase, B-glucuronidase, and N-acetyl-B-glucosaminidase) in various tissues of the rat, Hayashi (1964) has reported that although a close correlation exists for cellular sites of activity with respect to these three enzymes in most but not all tissues, the number of enzymatically active lysosomal particles and their

staining intensities do vary considerably with different tissues. In a limited biochemical survey of the distribution of lysosomal enzymes in animal tissues, Shibko, Caldwell, Sawant and Tappel (1963) have noted quantitative differences in lysosomal acid hydrolase content between tissues, and between species. The present study was undertaken in order to survey in greater detail the extent of heterogeneity of lysosomes in different tissues of a given species, and in homologous tissues of different vertebrate species, employing a battery of six cytochemical methods for acid phosphatase, B-glucuronidase, N-acetyl-B-glucosaminidase, and non-specific esterase. In all, twenty-seven species of mammals, birds, reptiles, amphibia, and fish were studied.

Before discussing the results of this comparative histochemical study of lysosomal enzymes, it is necessary to set out some of the basic tenets and problems that relate to a histochemical approach.

Validity of Histochemical Methods for Lysosomal Acid Hydrolases

The aim of enzyme histochemistry is the localization of specific enzymatic activities to intact tissue components *in situ* in histologic preparations. The characterization and semi-quantitation of the enzymic activity mediating the histochemical reaction is as integral a function of this discipline as the localization of the activities itself. As already indicated, the potential for histochemistry is great, inasmuch as it offers an excellent opportunity to relate cellular and intracellular structures to biochemical properties and physiologic functions. Unfortunately, the apparent simplicity of histochemical techniques tends to obscure the underlying complexity of many histochemical staining reactions.

General Considerations

The histochemist, unlike the biochemist, is mainly concerned

with the characterization of enzymes that are present in tissues in an insoluble state, or can be made so by a variety of denaturing techniques; this activity is then demonstrated in situ by the formation of a dense colored insoluble precipitate. Thus, the nature of enzyme histochemistry demands that, for an enzyme to be localized, it must be insoluble or insolubilized, and that to be characterized and quantitated it must be localized to morphologic structures (Pearse, 1960; Burstone, 1962; Barka and Anderson, 1963; Glenner, 1965; Hess, 1965).

I. Factors Affecting Enzyme Localization

There are few procedures that a tissue undergoes in the hands of the histochemist that are not potentially capable of affecting enzyme content and localization. A biochemical concept, that of the existence of cellular enzymes in an extractable, soluble "lyo-enzyme" form, as well in particulate, insoluble "desmo-enzyme" form has been reintroduced into histochemistry to explain the loss of specific enzymic activity from the tissue section into the histochemical incubation medium (Hannibal and Nachlas, 1959). In an attempt to reduce the diffusion of soluble enzymes from tissue sections during incubation, histochemists have sought to immobilize these enzymes by a variety of denaturing techniques, particularly fixation in cold dilute solutions of either formaldehyde or glutaraldehyde (Sabatini, Miller and Barrnett, 1964). However certain problems arise in interpreting the results obtained with denatured tissue. These include an assessment of the degree of enzymic inactivation resulting from fixation, the effect of fixation on the activity characteristics of enzymes or families of enzymes, preferential extraction of co-factors, altered penetration of substrate and capture reagent into organelles which have been modified by fixation, and the possible removal of endogenous

inhibitors (Barka and Anderson, 1963; Glenner, 1965). Holt and Hicks (1961) found approximately 90% inhibition of acid phosphatase activity in rat kidney and liver following fixation in 4% formol-calcium for 24 hours. This inhibition was minimized to 60%, if such fixation was followed by washing for 12 hours, and to about 40% if the tissue blocks were washed with gum sucrose at 2° C. for 24 hours. Accordingly, Holt's regime for fixation of tissue for the demonstration of acid hydrolases was used throughout in the present study. Different lysosomal acid hydrolases and their iso-enzymes vary somewhat in their susceptibility to fixation (Shnitka and Seligman, 1961). Notwithstanding the foregoing, treatment of tissue with fixatives is necessary for optimal preservation of morphologic structure. This is particularly true with regard to the preservation of intact lysosomes within cells and tissues.

The Mechanism of Histochemical Localization

In simultaneous-coupling histochemical reactions, enzyme contained within tissue sections hydrolizes a synthetic substrate with a specific chemical configuration for the enzyme, to yield an initial reaction product that in turn reacts with a capture reagent in a capture reaction to yield a final histochemical reaction product (Glenner, 1965). Ideally, the latter should be highly insoluble, colored, non-crystalline, and substantive for protein. Precision of localization of the reaction product can be impaired by diffusion of enzyme during fixation or in the course of the histochemical incubation, or by diffusion of the initial reaction product away from the enzyme site in tissue, or by diffusion of the final reaction product away from the enzyme site, particularly if the capture reaction occurs slowly (Barka and Anderson, 1963; Glenner, 1965). In the histochemical procedures for acid hydrolases used in the

present study, these sources of diffusion artifact have been largely obviated. False localization of reaction product may result from diffusion and relocation of enzyme within the tissue, diffusion of enzyme into the fixative or incubating medium, adsorption of diffusable enzyme to non-enzymatic sites, and through solubility of the final reaction product in tissue constituents, particularly lipids (Glenner, 1965). These considerations become important when distinguishing between particulate localization to lysosomes and diffuse localization in cytoplasm (endoplasmic reticulum localization).

Sources of Inhibition of Enzyme Activity in Histochemical Reactions

Although most enzyme histochemical techniques have been designed to provide optimal conditions for enzyme activity, often some compromise is unavoidable. In general, some enzyme activity is sacrificed for the sake of precise morphologic localization. In addition to enzyme denaturation through aldehyde fixation, enzyme activity in tissue sections may be further impaired by loss of activators or co-factors, non-optimal substrate concentration, non-optimal incubation temperatures, incubation at a non-optimal pH for the enzyme in order to facilitate the capture reaction, and the presence within the reaction mixture of enzyme inhibitors such as diazonium salts, lead or calcium ions. At present, there is no satisfactory way for determining the absolute sensitivity of a histochemical technique (Glenner, 1965).

The Effect of Iso-enzymes on Histochemical Procedures

Recent studies indicate that the occurrence of iso-enzymes of acid hydrolases may have considerable bearing on their histochemical localization (Shnitka and Seligman, 1961). Evidence derived from

electrophoretic, chromatographic, and immunologic investigations suggest that hydrolytic enzymes form families of closely related members. The various members (iso-enzymes) represent molecular varieties of the same enzyme. These iso-enzymes generally split the same substrates, but at different rates, and have close or identical pH optima. They differ in their electrophoretic mobility and have different Michaelis constants. (Barka and Anderson, 1963; Glenner, 1965). The use of electrophoretic (zymogram) techniques to characterize enzymes with histochemical reagents under histochemical conditions was initially developed by Hunter and Markert (1957), Hunter and Burstone (1960), and Allen and Hunter (1960). With the zymogram technique, no fewer than ten soluble non-specific esterases, all of which are capable of hydrolizing various esterase substrates, have been separated from mouse liver (Hunter and Burstone, 1960). Similarily, using anion and cation exchange cellulose chromatography and electrophoresis on acrylamide gel, at least four acid phosphatases had been separated from rat liver (Barka, 1961 a, 1961 b). Fixation, and the various other factors operative in a histochemical system, outlined previously, may selectively inhibit some iso-enzymes and thereby lead to a more conspicuous staining of the remaining hydrolase activity (Shnitka and Seligman, 1961). Histochemical localization therefore does not necessarily represent a summation of iso-enzyme activities in a tissue. Differing substrate affinities for iso-enzymes of acid hydrolases prompted the use in parallel of two histochemical methods for non-specific esterases and for acid phosphatase, that employ different substrates.

Enzymic versus Non-enzymic Methods for the Histochemical Demonstration of Lysosomes

Included in a recent review on the "Histochemistry of Lysosomes"

by Gahan (1967) are six non-enzymatic histochemical methods for the demonstration of lysosomes in tissue sections:-

1. Phospholipids

The method of Baker (1946, 1947) has been employed to detect lysosomal membranes by staining the phospholipid moiety of the membrane. This method is most useful when applied to rat kidney which contains large lysosomes, but tends to yield erratic results even with this material.

2. Polysaccharides

Koenig (1962) has reported that lysosomes in brain yield a positive staining reaction for polysaccharides. Because of the ubiquitous occurrence of polysaccharides in tissues, this procedure lacks specificity.

3. Ammoniated silver carbonate

Sandberg and Becker (1964) proposed the use of ammoniated silver carbonate as an emperic stain for lysosomes in rat brain and rat kidney. The reaction appears to depend in part on an affinity of metal salts for negatively charged protein in the matrix compartment of lysosomes and in part on oxidation of contained polysaccharides by silver cations in alkaline solution, leading to a deposition of metallic silver. In our experience, this method yields irregular results.

4. Autofluorescence

Koenig (1963) has reported that lysosomes in a number of rat tissues display autofluorescence when irradiated with ultraviolet light. This autofluorescence is difficult to distinguish from other sources of autofluorescence in tissue, however.

5. Vital Dyes

In tissue culture, living HeLa cells preferentially sequestrate dyes such as Acridine Orange and Euchrysin in lysosomes (Robbins and Marcus, 1963; Robbins et al., 1964). Mulnard (1961) has similarly

employed neutral red and brilliant cresyl blue as markers of lysosomes. Vital staining of lysosomes in the intact animal, however, is largely limited to phagocytic cells of the reticulo-endothelial system and to renal tubular epithelial cells.

6. Metachromasia

Lysosomes may show metachromatic staining with toluidine blue because of the presence of an acid mucopolysaccharide component within the matrix compartment of the organelle (Mulnard, 1961; Dalcq, 1963). Again, this staining technique lacks specificity because of the wide occurrence of metachromasia in stromal elements of tissue.

While under certain circumstances non-enzymatic histochemical techniques may serve to demonstrate the presence of lysosomes, these are often capricious, relatively non-specific, and lack the biochemical and functional significance that can be obtained through the use of enzymatic histochemical procedures for lysosomal acid hydrolases.

II. Factors Affecting Histochemical Quantitation of Enzyme Activity

During the past century, there has been a growing interest amongst biologists in the use of the microscope as an instrument for the analysis and quantitation of chemical constituents in cells and tissues, rather than as a strictly morphologic tool (Wied, 1966). Such approaches have now achieved a moderate degree of success in relation to the quantitation of dry mass of cells and particles, D.N.A. content, R.N.A. content, and the estimation of simpler tissue components such as sulfhydryl groups, acid mucopolysaccharides, lipid-containing structures and proteins (Wied, 1966). Unfortunately, in quantitative evaluation of a histochemical enzyme system, not only must factors affecting localization and characterization be considered, but also the complex

problems of enzyme inactivation and inhibition must be taken into account. Histochemical quantitation of an enzyme reaction requires that the substrate concentration be sufficiently high to ensure that the liberation of initial reaction product follows zero-order kinetics. The initial reaction product thus liberated must be quantitatively precipitated by the capture reagent and also quantitatively transformed to a final reaction product. The reaction product must not be inhibitory to enzyme activity at the site of precipitation. Usually, because of losses incurred through fixation and leaching. The enzyme activity preserved in tissue sections represents only a portion of the total initial activity (Glenner, 1965). With these limitations in mind, histochemists frequently speak in terms of "more" or "less" activity in various tissue sites. It is also a common practice to evaluate subjectively deposits of reaction product in tissue sections on a one-to-four grading scale (Woessner, 1965; Hayashi, 1967). In the present study in order to achieve some measure of semi-quantitation, conditions of incubation for each of the histochemical reactions employed were standarized, and incubation periods were kept short. The size, and population density of stained lysosomes in various locations were also graded, in order to permit rough comparisons with data from biochemical literature. The problems of quantitation of histochemical methods have been discussed in detail by Glenner (1965).

III. Histochemical Evidence for Homology and Heterogeneity of Lysosomes in Different Tissues of the Same Species and Homologous Tissues of Different Species

A. Comparative Histochemistry of Lysosomal Acid Hydrolases in Different Tissues of the Same Species

By way of generalization, in the present histochemical survey of distribution of lysosomal acid hydrolases in six standard tissues of twenty-seven vertebrate species, lysosomes are particularly numerous in cells of proximal tubules of kidney, intercalated cells of renal collecting tubules in mammals, hepatic parenchymal cells and Kupffer cells, acinar cells of pancreas , macrophages of red and white pulps of spleen, macrophages of lamina propria of small intestine, absorptive epithelial cells of villi of small intestine, cortical neurones of cerebrum, Purkinje cells of cerebellum, and perithelial microglial cells of brain. These observed sites of high activity of lysosomal acid hydrolases are in agreement with the findings in rat tissues reported by Hayashi (1967), and the biochemical distribution of six lysosomal enzymes in approximately ten species of animals noted by Shibko, Caldwell, Sawant and Tappel (1963). Minor sites of lysosomal acid hydrolase activity in the present study include distal renal tubule, renal collecting ducts, Islets of Langerhans of pancreas, pancreatic duct epithelium, lymphoid cells of white pulp of spleen, glial cells of the brain, and vascular endothelial cells of the brain.

Histochemical distributions and relative activities of acid phosphatase, non-specific esterase, glucosaminidase, and glucuronidase, for the twenty-seven vertebrates species studied are shown in the accompanying tables.

As noted by Hayashi (1967) in his histochemical survey of the distribution of three lysosomal acid hydrolases in various tissues of the rat, acid phosphatase activity is high in numerous lysosomes in all

of the sites enumerated above. Observed differences in staining intensities obtained with Gomori's method and Barka's method for acid phosphatase in some tissues of the rat, probably reflect differences in the distribution of iso-enzymes of acid phosphatase. Naphthol-AS TR phosphate may be split at a somewhat slower rate than is B-glycerophosphate, because of steric hindrance. Four acid phosphatase iso-enzymes have been noted in zymograms of rat liver (Barka, 1961a, 1961b). Non-specific esterase activity localized to lysosomes has a similar tissue distribution to acid phosphatase, in the rat. Non-specific esterase, more than any of the other hydrolytic enzymes studied shows intense diffuse cytoplasmic staining in addition to lysosomal staining, particularly in epithelial cells of liver and kidney (Shnitka and Seligman, 1961). Such diffuse cytoplasmic staining probably reflects iso-enzyme activity in non-lysosomal cytoplasmic components (i.e., endoplasmic reticulum). In tissues of the rat, the two glycosidases studied (B-glucuronidase and glucosaminidase) tend to be considerably more limited in their distribution than acid phosphatase and variable in their intensity of staining with respect to acid phosphatase, and with each other. Glucuronidase activity is of moderate intensity in proximal convoluted tubules of kidney,while glucosaminidase shows intense activity at the same site. In parenchymal cells of the liver, glucuronidase has intense activity while glucosaminidase is absent. In the spleen, macrophages carry abundant glucuronidase activity while glucosaminidase is low or absent at this site. Surprisingly, glucuronidase activity is very scant in absorptive epithelium of small intestine. Cortical neurones and Purkinje cells of the rat brain are also deficient in glucuronidase activity; there is slight glucosaminidase activity in lysosomes of these cells. Perithelial microglial cells contain an abundance of both

enzymes. Hayashi (1967) reports further qualitative differences in the distribution of these two glycosidases in several tissues not examined in this study, including adrenal gland, squamous epithelium of the tongue, esophagus, and preputial gland. Scrutiny of histochemical data for other species in Tables I and II discloses a number of patterns of variation of individual lysosomal hydrolases in tissues of other species of animals.

In most mammalian species studied, the tissue distributions of acid phosphatase activity, and of lysosomal and diffuse cytoplasmic, non-specific esterase activities parallel those of the rat. Enzyme activities are high in tissues with large numbers of lysosomes. The sheep shows abundant acid phosphatase activity in proximal convoluted tubules, but very sparse activity in the liver, largely limited to Kupffer cells. Cortical neurones and Purkinje cells of the sheep are also devoid of acid phosphatase activity. In the field mouse, as opposed to the Ajax strain of the laboratory mouse, acid phosphatase shows low activity in parenchymal cells of the liver, but high activity in Kupffer cells. In the various species of mammals studied, glucuronidase and glucosaminidase show a more restricted distribution and a greater quantitative variation in tissues, than acid phosphatase. The rabbit contains very high glucuronidase activity in parenchymal cells and Kupffer cells of the liver, but virtually no glucuronidase activity in the kidney. Glucuronidase is also absent from cells of the brain, in the rabbit. The pig lacks glucuronidase in proximal tubules of kidney, and has only low activity in liver and in splenic macrophages. In man, there is moderate glucuronidase activity in lysosomes of proximal tubules of kidney, parenchymal and Kupffer cells of the liver, splenic

macrophages, and macrophages of lamina propria of small intestine. With respect to glucosaminidase activity, both kidney and liver contain an abundance of this enzyme, as do macrophages of spleen, and cortical neurones and perithelial microglial cells of brain. Proximal tubules display moderate glucosaminidase activity in lysosomes, but most other tissue sites are devoid of significant activity, in man. These tissue variations are best appreciated through a direct scrutiny of the data provided in Tables I and II.

The five species of birds examined show a pattern of distribution of lysosomal acid phosphatase very similar to that encountered in mammals. There is high activity in cells of proximal tubules, hepatocytes, Kupffer cells, absorptive cells of duodenum, macrophages of red and white pulps of spleen, and of lamina propria of duodenum, and in cortical neurones, Purkinje cells and perithelial microglial cells of brain. The iso-enzymes of non-specific esterase (with substrate affinities respectively for a-Naphthyl acetate and Naphthol AS-acetate)in general parallel the distribution of acid phosphatase in tissues of birds, but display low activity in Kupffer cells, macrophages of spleen, macrophages of lamina propria of small intestine, and cortical neurones. In birds, B-glucuronidase activity is high in proximal convoluted tubules of kidney, moderately active in hepatic parenchymal cells and macrophages of spleen and lamina propria of small intestine, but of very low activity in cortical neurones and Purkinje cells. The horned owl shows very low glucuronidase activity in most tissues including kidney, liver, spleen, intestine and brain. The distribution of glucosaminidase in birds is somewhat more variable. There is high activity in proximal tubules, Kupffer cells and perithelial

microglial cells; low activity exists in hepatocytes. In the Japanese quail there is considerable activity in absorptive epithelium of duodenum, while in the horned owl activity for this enzyme is low.

In the four species of amphibia examined (grass frog, tree frog, newt and salamander), there is generally very high acid phosphatase activity in proximal tubules of kidney, hepatic parenchymal cells, Kupffer cells of liver, macrophages of spleen, absorptive epithelium and macrophages of duodenum, cortical neurones and perithelial microglial cells of brain. In the brain there is some variability in acid phosphatase activity between species with most intense staining in the grass frog, and scant or absent staining in the tree frog and newt. Non-specific esterase, follows an almost parallel distribution to acid phosphatase throughout tissues of amphibia, with the exception of all cells of the brain which are deficient in this enzyme. Glucuronidase activity in lysosomes of amphibia is variable, with only moderate activity in proximal renal tubules and moderate to intense activity in hepatocytes, Kupffer cells, and splenic macrophages; activity is slight or absent in the duodenum, and in cells of the brain. Glucosaminidase activity in lysosomes of amphibia presents an almost identical pattern of distribution.

In the two species of reptilia examined (chameleon and turtle) there is only modest acid phosphatase activity in proximal renal tubules and in liver; in the chameleon high acid phosphatase activity exists in macrophages of spleen and in absorptive cells of duodenum. There is high acid phosphatase activity in cortical neurones of the turtle. Non-specific esterase activity in the chameleon and turtle are similar in distribution and intensity of staining to acid phosphatase, except that in parenchymal cells of the liver activity tends to be quite low.

Esterase is absent in all cells of the brain of both species. Glucuronidase is absent from all tissues of the chameleon. The liver of the turtle contains moderate activitiy of this enzyme in parenchymal cells , and Kupffer cells. Other sites are low or negative. With respect to glucosaminidase activity, all tissues of the chameleon are negative. In contrast, the liver and kidney of turtle show moderate activity. There is also moderate glucosaminidase activity in splenic macrophages and cortical neurones and perithelial microglia cells of turtle.

B. Comparison of Similarities and Differences in Size and Population Density of Lysosomes in Homologous Tissues of Different Vertebrate Species

Lysosomes as observed in histochemical preparations and in electron micrographs range in diameter from 0.3 to 7 microns. In addition, lysosomes vary considerably in number in diverse cells and tissues. Large lysosomes usually possess high specific acid hydrolase activity, particularly for acid phosphatase and esterase. In individual cells, large lysosomes tend to be few in number, while small lysosomes tend to be numerous. Larger lysosomes with high acid phosphatase and esterase activities occur in macrophages of red and white pulps of spleen, and of lamina propria of small intestine, and in Kupffer cells of liver. Lysosomes of moderate size occur in proximal renal tubules and in cortical neurones. Small lysosomes are distributed in the collecting tubules and the collecting ducts of kidney, in hepatic parenchymal cells, pancreas, and absorptive cells of the duodenum. In proximal renal tubules, differences is size of lysosomes are observed among species. With Gomori's acid phosphatase method, lysosomes of proximal tubules of rat kidney are large (3 to 7 microns in diameter); those of the field mouse, cow,

sheep, cat and man are small (0.5 to 3 microns). Lysosomes appear to be of large size in those tissues that are actively engaged in either phagocytosis or pinocytosis. In this context it is of interest that adult male rats develop a physiologic proteinuria accompanied by resorption of protein in proximal tubules, which is not manifest to the same extent in other species (Logothetopoulos and Weinbren, (1955).

Lysosomes within single cells of similar type may vary in enzyme content. When a battery of histochemical stains is applied to serial sections of some tissues, glucosidase activities tend to be localized to small lysosomes, whereas, acid phosphatase and esterase activities prevail in the larger lysosomes.

C. Dual Lysosomal and Microsomal Histochemical Localization of Acid Hydrolases in Certain Sites

Histochemical staining reactions for non-specific esterase in parenchymal cells of liver and kidney of most species show both lysosomal and diffuse cytoplasmic staining. The latter is considered to be a valid expression of enzyme activity inasmuch as cytoplasmic staining does not occur in other tissues, under similar experimental conditions. This phenomenon was studied extensively by Shnitka and Seligman (1961) who concluded that two families of iso-enzymes of esterase are involved, one in endoplasmic reticulum that is sensitive to inhibitors (such as sodium fluoride, arsanilate and diisopropyl-fluorophosphate) and the other located within lysosomes which is resistant to inhibitors. It is of significance that aldehyde fixation may selectively inhibit the inhibitor-sensitive cytoplasmic esterase, and leave intact the lysosomal iso-enzyme. In the case of esterase activity, it seems likely that the iso-enzyme(s) associated with endoplasmic reticulum subserve a different

physiologic function than those located in lysosomes. Lysosomal esterase activity is still an enigma, and may represent an expression of esteratic activity of proteolytic enzymes, such as cathepsins C (Hess, 1965).

In the present study, the histochemical reaction for glucuronidase activity shows both particulate lysosomes staining and diffuse cytoplasmic staining in hepatic parenchymal cells of the hamster, mouse (Ajax), cow, and Japanese quail. Fishman, et al., (1967), have recently correlated B-glucuronidase staining reactions of intact tissues with the activity of subcellular fractions of tissue homogenates. As these authors point out, in a given system, intensity of staining should reflect the extent of the enzyme activity, which in turn should be demonstrable in the subcellular fraction that corresponds to the organelle of cytoplasm which stains positively. In tracer studies involving the incorporation of carbon-14-labeled leucine into B-glucuronidase of mouse kidney, under androgenic stimulation newly produced enzyme first appears in endoplasmic reticulum (microsomal fraction), and later in lysosomes. This suggests that B-glucuronidase is initially synthesized in the endoplasmic reticulum and is subsequently concentrated in lysosomes. Ganschow and Paigen (1967) have recently reported that separate genes determine the structure and intracellular location of hepatic glucuronidase in different strains of inbred mice. Normally, glucuronidase resides within both lysosomes and membranes of the endoplasmic reticulum in murine liver cells. Enzyme molecules resident at these two morphologic sites are indistinguishable by physical methods of enzyme separation. A mutant strain of inbred mice (YBR-strain) displays a marked reduction of B-glucuronidase activity in liver cells. The enzyme is

deficient in endoplasmic reticulum but is retained in lysosomes. The loss of endoplasmic enzyme is attributed to a mutation of a Mendelian gene which is distinct from the glucuronidase structural gene. The new gene, symbolized as E, does not appear to affect other endoplasmic proteins and is tissue specific for liver. These results indicate that the intracellular localization of acid hydrolases may be under gene control.

In the present study, with Barka's Naphthol-AS method, diffuse cytoplasmic staining for acid phosphatase is occasionally noted in liver cells of mice of the Ajax strain, and in intestinal absorptive epithelium and cortical neurones of the rat. From biochemical studies, Nelson (1966) has reported that acid phosphatase activity of rat liver is primarily lysosomal, with 70 to 80% of total activity located in this organelle. The remainder, however, is distributed in the cytoplasm. Hepatic acid phosphatase of lysosomal origin is very sensitive to L-tartrate and flouride inhibition, while hepatic acid phosphatase from the supernatant (endoplasmic origin) is resistant to the foregoing inhibitors, but is inactivated by exposure to heat (i.e., 50° C. for 20^{+} minutes).

With the Naphthyl AS-acetate method for non-specific esterase, diffuse cytoplasmic staining often is associated with a fine granularily of the deposited reaction product. This is generally regarded as an artifact of the method, produced by crystallinity of the reaction product. Crystallinity is partially obviated by incorporation into the reaction medium of solvents such as propylene glycol or acetone.

D. Patterns of Tissue Distribution of Lysosomal Enzymes in Different Species

Comparative enzyme biochemistry has received relatively little attention, although presently there are scattered reports in the literature concerning differences in enzyme content of homologous organs of different species. Dixon and Webb (1964) have compiled much of this data in tabular form in their treatise on the "Enzymes" (see Appendix). Comparative enzyme biochemistry encompasses two distinct fields of study, namely, (a) a comparison of the enzymatic equipment of organs of different species, as in the present study, and (b) a comparison of pure preparations of a given enzyme obtained from different animal sources. Data compiled by Dixon and Webb (1964) show surprisingly large quantitative differences among tissues and species for given enzymes. High activity of a number of enzymes appears to be characteristically associated with particular organs. For example, D-amino acid oxidase, xanthine oxidase and catalase are present predominantly in liver and kidney, but are practically absent from muscle. Alkaline phosphatase is abundant in intestinal mucosa, acid phosphatase in kidney and spleen, galactosidase and mannosidase in epididymus, and ribonuclease in pancreas. As more information becomes available, these differences will probably fall into recognizable patterns of metabolic specialization of individual organs. Another suggestion is that these differences are correlated with the size of the animal; the rat and mouse which have a high metabolic rates because of their small body size, contain larger amounts of many enzymes per unit weight of tissue than do the larger animals. This however, is by no means an invariable rule. In comparing lysosomal acid hydrolases in lysosomal fractions obtained from livers of rat and sheep, Shibko, Caldwell, Sawant and Tappel (1963) noted high acid phosphatase in the rat, low acid phosphatase in sheep, an absence of galactosidase in rat,

slight activity in sheep, high acid ribonuclease activity in both rat and sheep, scant arylsulphatase activity in the rat, very high arylsulphatase activity in the sheep, high B-glucuronidase activity in the rat and moderate B-glucuronidase activity in the sheep.

In comparing the spectrum of acid hydrolase in sheep lung macrophages with that of sheep liver, the same authors noted a factorial difference of 50 between the lysosomal B-galactosidase content of liver versus that of lung. Five other lysosomal hydrolases were of comparable activity in the two organs. Considering whole tissue homogenates, Woessner (1965) reviewed data from the literature concerning the distribution of lysosomal acid hydrolases in liver and spleen of mouse and rat, again with quantitative differences between species. However, it should be cautioned that these data are derived from different sources and in part reflect values obtained by different methods. Most animal tissues include multiple cell types, differing enzyme content. Accordingly, biochemical experiments utilizing homogenates of whole tissues merely provide an average of the enzyme content of all of the cell types present. Some biochemical workers have attempted to subdivide tissues by dissection; for example, enzyme determinations have been made on intestinal mucosa instead of the whole intestinal wall, on renal cortex and medulla instead of whole kidney, and on grey and white matter, instead of whole brain. These separations, however, are relatively crude and of little use in circumstances where cell populations are intimately admixed. Also, it is by no means certain that even cells which are histologically of the same type have the same enzyme composition. Novikoff, in a histochemical study, has noted that there are gradients of lobular activity in liver, for many enzymes. Lysosomal hydrolases

in liver tend to be predominantly of centrilobular distribution.

Apart from the quantitative differences in enzyme content discussed above, qualitative differences in enzyme proteins also exist between organs and species. These are best demonstrated by the use of zymograms. This approach has been extensively applied to the characterization of non-specific esterases and lactic dehydrogenases. (Markert and Moller, 1959; Markert and Hunter, 1959; Markert and Ursprung, 1962; Barron et al., 1962; Pesce et al., 1963; Ecobichon, 1967). Iso-enzymes of esterase show marked organ and species differences (Ecobichon, 1967).

Also, an enzyme may exist in different crystalline forms when purified from different animal species. Examples can be found in the atlas compiled by Dixon and Webb (1964). The foregoing reflect inter-species variations in amino acid composition of iso-enzymes,which are capable of producing significant differences in kinetics or specificity of an enzyme reaction.

Histochemical methods for acid hydrolases permit the detection of cells with high enzyme activity, in a mixed population of cells. However, for various reasons outlined previously, only crude semi-quantitative results can be obtained by histochemical techniques. All-or-none differences, however, are discernible in histochemically stained preparations, and these provided meaningful results because of the precision of localization achieved and the ease with which diverse tissues can be surveyed. Gahan (1967) and Woessner (1965) have reviewed in tabular form fragmentary data concerning the distribution and semi-quantitation of lysosomal hydrolases in tissues of various vertebrate species. There are many deficiencies, however, in these tables and some of the tabulated findings are based on extrapolations from published photomicrographs. In the present study, there appears to be

considerable heterogeneity with regard to enzyme content of lysosomes from different organs. While acid phosphatase tends to be high in most major locations of lysosomes, companion enzymes, particularly B-glucuronidase and glucosaminidase are not necessarily present in similar amounts in lysosomes of different organs. Even within different segments of renal tubules, lysosomes show variations in histochemical staining patterns for acid hydrolases. Thus, lysosomes in proximal convoluted tubules of hamster kidney show very low glucuronidase activity, but high glucuronidase activity in intercalated cells of collecting tubules. The same type of quantitative difference is observed in guinea pig kidney. Glucuronidase activity is totally absent from all segments of the nephron of the field mouse, while glucosaminidase is absent from renal tubules of the rabbit. Both, of these species, however, show high acid phosphatase activity in proximal tubules and intercalated cells. Thus, patterns of histochemical staining for lysosomal hydrolases often tend to be "organ-specific" in individual species. Macrophages of red and white pulps of the spleen, lamina propria of small intestine, and perithelial microglial cells of brain, show similarities in staining in a given species, in keeping with the "reticuloendothelial system concept" proposed many years ago by Aschoff (1924).

From the present study no generalizations can be made concerning characteristic phylo-genetic patterns of lysosomal acid hydrolase activity. Liver and kidney of most vertebrates are rich in lysosomal acid phosphatase and in non-specific esterase. Glucuronidase and glucosaminidase tend to be more variable and of restricted distribution in tissues. Acid phosphatase activity is high in amphibia and fish, in liver,

kidney, spleen and small intestine. Species differences in acid hydrolase content within phyla and the limited number of species studied, preclude generalization concerning phylogenetic patterns. Also, in some circumstances it is difficult to compare homologous tissues of species from different phyla because of histologic differences in organ structure, which may entail the presence or absence of specific cell types (Andrew, 1959). For example, intercalated cells appear to be absent from kidneys of birds. Fish possess a mesonephric kidney, with abundant lymphoid and erythropoietic tissue in renal stroma (Good, Finstad, Pollara, and Gabrielsen, 1966).

Shibko, Caldwell, Sawant and Tappel (1963) have included in their biochemical survey of lysosomal enzymes in animal tissues, primitive animals such as snails, clams, crustacea and insects. All of these appear to carry the same acid hydrolases as are identified in mammalian tissues. Presently, there are no discernible patterns of evolutionary change in enzyme content of lysosomes. Deficiencies of specific lysosomal hydrolases in certain tissues and species may reflect gene-controlled enzyme deletions through mutations, or effects of repressor genes, tailored to specific metabolic requirements.

Acid phosphatase has been used as a "marker" enzyme in both biochemical and histochemical studies of lysosomes in diverse tissues. This trend was established by Novikoff (1959c) on the basis of observed high lysosomal acid phosphatase activity in rat liver and kidney. While such an approach appears to be valid for lysosomes in liver, kidney and the reticulo-endothelial system of many vertebrate species, there are a few examples cited by Hayashi (1967) and by Shibko et al. (1963) where glycosidases occur in high activity but acid phosphatase activity is low.

In the present study, acid phosphatase activity is low or absent in Kupffer cells and hepatic parenchymal cells of sheep, whereas, non-specific esterase and glucuronidase are active in both of these sites; glucosaminidase is high in Kupffer cells only. In view of the heterogeneity as to enzyme content of lysosomes in different tissues and species, it would appear appropriate to use a battery of biochemical or histochemical methods for the characterization of lysosomes, rather than a single marker enzyme. If one is restricted to the use of a single enzyme, acid phosphatase would appear to be the most ubiquitous.

Lysosomes are thought to be involved in the digestion and storage of extra-cellular materials taken in by cells. Ingested materials include whole cells, sub-cellular particles, bacteria, viruses, and small molecules. The categories of substrates acted upon by lysosomal acid hydrolases encompass nucleic acids, proteins, glycogen, and mucopolysaccharides. However, in most circumstances, specific details are lacking concerning the precise natural substrates involved. For example, the natural substrates for acid phosphatase and for non-specific esterases are not known. Variations in enzyme content of lysosomes in different tissues and species, particularly with respect to glycosidases, may be a reflection of specialized function, or a limited handling of certain substrates.

As will be discussed presently, in the Experimental Section of DISCUSSION, a number of alterations in physiologic state are capable of producing modulations in the size, number and enzyme content of lysosomes. A well-studied example is the increase in population density and acid phosphatase content of lysosomes in proximal renal tubules of rat, which show a considerable increase in number and enzyme activity in male

rats, following puberty. The foregoing is related to physiologic proteinuria that occurs in the adult male rat (Logothetopoulos and Weinbren, 1955). The latter phenomenon is testosterone-dependent, and can be reversed in the adult male rat by castration, and induced in the female rat by the administration of testosterone following castration. Sex, age, and starvation may affect the size, number and enzyme content of lysosomes, but these physiologic factors are as yet poorly studied. In the present study, it was in some instances difficult to obtain large numbers of animals of a given species, so that no comment can be made specifically with regard to effects of age and sex on acid hydrolase content of tissues.

IV. Histochemical Evidence for Homology and Heterogenity of Lysosomal Acid Hydrolases in Tissues of Different Species

Among animals, irrespective of their taxonomic position, one encounters biochemical similarities that indicate a unity of biochemical design in animal organization. These biochemical similarities shared by animal cells of diverse origins permit a large number of generalization concerning intermediary metabolism. While it is true that animals manifest a remarkable unity of composition, further detailed examination of their cellular constituents or of the relative proportions of the foregoing, brings to light certain qualitative and quantitative biochemical differences between tissues and species. These differences comprise the subject material of comparative biochemistry, and have been reviewed by Florkin (1949) and Anfinsen (1963) in relation to respiratory function, hydrolytic processes of digestion, protein metabolism, and purine metabolism. A number of proteins derived from different vertebrate species, including ribonuclease, adrenocorticotropin,

melanotropin, insulin, cytochrome-C, hemoglobin and serum albumin show major chemical similarities, and also minor differences in amino acid composition and sequences (Anfinsen, 1963). These are thought to be on the basis of genetic control, species differences arising through mutations that have occurred during the course of evolution. In this context, Neurath et al. (1967) have recently reviewed the evolution of structure and function of proteases. These authors consider phylogenetic relationships among enzymes in terms "analogies" and "homologies". Analogy is used to denote similarities in enzyme function without regard to protein structure. Three types of analogy may exist; (1) analogy in enzymatic function (such as substrate specificity); (2) analogy of enzymatic mechanisms; and (3) analogy in biological properties. Enzymatic function relates to the nature of the chemical bond in the substrate that is being split, and therefore is an expression of specificity of the enzyme site. Analogy of enzymatic mechanism relates to physical-chemical features of the active enzyme site that are concerned with the bond-breaking mechanism after formation of the enzyme-substrate complex. Biologic analogies of enzyme function refer to such properties as tissue of origin, site of action in tissue, or physiological significance. The present study is largely concerned with analogies of enzymatic function and analogies in biochemical properties of lysosomal acid hydrolases. The term homology as applied to enzymatic proteins refers to a similarity in amino acid sequence. This can only be established through detailed chemical analysis, and the construction of peptide maps (Anfinsen, 1963; Neurath, 1967). The value of the foregoing classification is to provide a conceptual scheme for the genetic control of enzymic proteins. In relation to proteases, Neurath et al. (1967) present evidence to indicate

that gene duplication has led to the modification of enzyme specificity without affecting the enzymic mechanism, while minor variations in enzymic structure resulting from allelomorphism provide a mechanism for divergent evolution and modulation of a particular enzyme, by providing new variants of a particular enzyme in emerging species.

Discussion of Modulations in Lysosomal Enzymes Produced by Experimental Means

Considerable evidence is now available that lysosomes within cells participate in repeated digestive events involving endogenous and exogenous materials, and that these organelles are in a constant state of turnover. deDuve (1963) has estimated that approximately 3% of lysosomes in rat liver are lost and renewed per day. The origin of lysosomes is still controversial. Novikoff and co-workers (1964) have postulated that pinocytosis vacuoles may be supplied with hydrolases from Golgi vesicles, and in addition that areas of endoplasmic reticulum situated in the Golgi region are involved in the transfer of enzymes from the endoplasmic reticulum to the Golgi apparatus, thereby giving rise to lysosomal particles directly. In addition, lysosomes may undergo a number of structural-functional changes during their cycle within cells, that include transformation from primary lysosomes to phagolysosomes of cyto-lysosomes, and finally to residual bodies. Changes in enzymatic content probably accompany the foregoing alterations. During the past decade, a number of experimental models have been described wherein acid phosphatase and other lysosomes hydrolases undergo quantitative changes in liver and kidney of rat and mouse in response to agents that produced alterations in lysosomes. Inasmuch as biochemical values represent mean values derived from large populations of lysosomes within tissues, it is not clear from these published results, whether quantitative modulations in lysosomal acid hydrolases result from an increase in size, or number, or specific activity or from enzyme inhibition. Histochemical techniques permit a high degree of precision of localization of acid hydrolase activity. On this basis, a number of

published experiments involving alterations in lysosomal enzyme through different mechanisms have been repeated in the present study, and histochemical staining results are compared with previously published biochemical values. Through such an approach, it was hoped that information could be obtained concerning the mechanisms whereby levels of acid hydrolases are altered by experimental manipulations.

I. (A) Hormone Induction of a Lysosomal Enzyme

(1) Effect of Testosterone Propionate on Glucuronidase Activity in Mouse Kidney

In a series of comprehensive papers published between 1955 and 1958, Fishman and collaborators reported that certain steroids, particularly those closely related to testosterone, possess the ability to enhance B-glucuronidase activity in kidney of various strains of mice. Fishman, Artenstein and Green (1955) established experimental conditions which made it possible to obtain uniform and reproducible values for renal B-glucuronidase activity in the Ajax strain of mice. A large number of steroids were screened by these workers. Androgens as a group were found to increase renal B-glucuronidase. Testosterone produced a 3-fold increase in renal glucuronidase activity, in the male mouse, whereas testosterone propionate produced a 16-fold increase. No other tissues, responded to androgen stimulation in a similar fashion. Further extensive experimentation (Fishman, 1961; Fishman and Goldman, 1965) indicated that renal B-glucuronidase in mice is dependent on the strain of animal utilized, can be neutralized in vivo by the administration of estrogen, and is excreted in the urine in parallel with the levels achieved in renal tissue. This renal B-glucuronidase response appears to correlate best with the protein anabolic activity of specific

androgens. The increase in enzyme activity reflects an actual increment in enzyme protein in kidney.

In the present study, testosterone propionate produced a marked increase in the intensity of histochemical staining of B-glucuronidase in proximal convoluted tubules of male mice of the Ajax strain. Increased activity was observed both diffusely throughout the cytoplasm of these cells as well as in lysosomes. The latter did not appear to be appreciably increased in number. As in control animals, glucuronidase activity was restricted to proximal convoluted tubules and to intercalated cells of collecting tubules. There was no apparent proximal or distal spread of enzyme activity in the nephron. Fishman et al., (1967) have recently suggested that androgen stimulates the production of B-glucuronidase in the endoplasmic reticulum of renal tubular cells, and that subsequently the enzyme is transferred to lysosomes of these cells. The histochemical findings in the present study are consistent with this suggestion. Control animals treated with olive oil alone showed considerable depression of glucuronidase activity in renal tubular epithelium. There was no histochemical alterations in glucuronidase activity in mouse liver following the administration of testosterone propionate. As shown in this experiment, histochemical staining methods permit semi-quantitation when there is a very marked increase in enzyme activity.

(2) Effect of Oestrone on Glucuronidase Activity in Mouse Liver

In 1949, Kerr, Campbell and Levvy reported that the injection of 1.7 to 2.0 mg./Kg. of Oestrone into intact male mice produces a moderate elevation of hepatic B-glucuronidase activity. In repeating this experiment using a histochemical assay technique for B-glucuronidase,

no significant increase in activity was observed. This may either reflect a relatively low inherent sensitivity of the histochemical assay technique, or a lack of reproducibility of the results obtained by Kerr, et al. (1949). The latter is a distinct possibility inasmuch as their work has not been extended by Fishman and his collaborators (1958).

(B) Induction of Lysosomal Hydrolases Through the Formation of New Lysosomes

(1) By an Agent That Induces Pinocytosis and the Formation of Phagolysosomes

Van Duijn, Willighagen and Meijer (1959) and Meijer and Willighagen (1961), have reported, on the basis of both biochemical and histochemical determinations, that the intra-peritoneal injection of dextran solutions in mice results in an approximately 2-fold increase in both acid phosphatase and B-glucuronidase activity in liver within a period of 3 to 4 days. Dextran is known to be stored in lysosomes of Kupffer cells and hepatocytes where it is slowly degraded. Uptake into these cells is by pinocytosis. Increase in enzyme activity reported by the Dutch group of workers probably reflects an accelerated rate of formation of phagolysosomes in hepatic parenchymal cells, following the saturation of Kupffer cells with dextran. In repeating this experiment, no histochemical differences were observed in activities of acid hydrolases in hepatocytes or Kupffer cells, of test and control animals. Differences in strains of mice employed, spacing and duration of pulse-doses of dextran, and in methodology, may account for this discrepancy in results. Biochemical data published by Meijer and Willighagen (1961)

indicate that the magnitude of the effect is small.

(2) Induction of Lysosomal Acid Hydrolases Through the Production of Cytolysosomes Following the Administration of Glucagon

The discovery by Ashford and Porter (1962) that glucagon induces the formation of autophagic vacuoles in perfused rat liver prompted Deter and deDuve (1967) to subsequently undertake a combined biochemical and morphologic study of this phenomenon. These authors report that within 30 minutes after the injection of glucagon, cytolysosomes exhibit an increase in number. At the same time, their sedimentation characteristics undergo complex changes. There is also a 10 to 16% increase in acid phosphatase, a lesser increase in cathepsin D, and a barely detectable increase in acid deoxyribonuclease. All these modifications are maximal between 45 and 90 minutes and disappear by 4 hours. Correlative electron micrographs are shown in the appendix of this dissertation. In the present study, using histochemical assay methods, no detectable alterations were observed in activities of acid phosphatase, non-specific esterase, B-glucuronidase, or glucosaminidase, in liver. Again, these negative results must be taken as evidence of low sensitivity of histochemical methods for small biochemical changes.

II. Inhibition of Lysosomal Acid Phosphatase In Vivo

In 1967, Shibko reported that the oral administration to rats of a single dose of N-2-fluorenylacetamide (50 mg. per 100 gm. body weight) results in a marked decrease of lysosomal acid phosphatase activity. This decrease commenced 8 hours after administration of the compound, and reached a maximum by 16 hours. Acid phosphatase activity remained depressed for five days, and returned to normal during the

subsequent two days. This effect appeared to be limited to lysosomal acid phosphatase of the liver and kidney; acid phosphatase activity was not depressed in the spleen or in the intestinal mucosa. Other lysosomal enzymes, namely, arylsulphatase, acid ribonuclease and cathephsin were not affected by this treatment, nor were enzymes characteristic of other sub-cellular fractions, including glutamic dehydrogenase, uricase, catalase, and lactic dehydrogenase. The soluble fraction of cytoplasm of liver from N-2 fluorenylacetamide-treated rats, did not inhibit lysosomal acid phosphatase from normal rat liver, in vitro. The foregoing results, suggested to the authors, that N-2-fluorenylacetamide acts by specifically inhibiting the endogenous production of lysosomal acid phosphatase in vivo.

In the present study, histochemical staining techniques showed an approximately 50% reduction in acid phosphatase activity in renal tubular epithelial cells and in absorptive cells of duodenal villi, following treatment of rats with a single dose of N-2-fluorenylacetamide. Slight to moderate degrees of inhibition of acid phosphatase activity were observed in hepatic parenchymal cells. No changes were observed in other lysosomal enzymes. Inhibition of activity in duodenal mucosal epithelium was not recognized by Shibko (1967), in his biochemical study. This is explicable on the ground that biochemical values for acid phosphatase in duodenal mucosa are contributed both by surface epithelial cells, and by macrophages in the lamina propria. Activity in the latter cells does not appear to be impaired by N-2-fluorenylacetamide treatment. The findings observed herein raise the interesting possibility that in intact lysosomes, contained enzymes may require constant renewal through synthesis.

In a similar context, Barka, Schaffner, and Popper (1961) have reported a marked decrease in acid phosphatase activity in rat liver, spleen, thymus, and lymphnode following repeated intravenous administration of trypan blue. This colloidal dye appears to act as an inhibitor of acid phosphatase in phagolysosomes.

III. Effects of Drugs on Histochemically Demonstrable Lysosomal Acid Hydrolases

(A) Cortisone Treatment

Since membranes bounding lysosomes appear to resemble the plasma membrane, and since both are of lipoprotein nature, Weissmann (1965) has reasoned that a number of membrane-active drugs, vitamins, toxins, or other substances can act either to labilize or stabilize the lysosome. Evidence from various sources in support of the foregoing is reviewed by Weissmann (1965). Only a few agents that stabilize lysosomal membranes against injury have thus far been discovered. Foremost among these are the anti-inflammatory steroids, i.e., cortisone, cortisol, prednisone, and prednisolone. These anti-inflammatory steroids appear to act both in vitro and in vivo. They have been shown to stabilize lysosomes in vitro against excess Vitamin A, streptolysin, ultraviolet irradiation, progesterone, carbon tetrachloride, or incubation at varying pH or at different temperatures. Similarly, corticosteroids protect lysosomes of living cells to the labilizing effects of endotoxins, antigen-antibody reactions, excess oxygen, shock, ultraviolet irradiation, and Vitamin A intoxication.

For purposes of the present study, cortisone acetate was administered in high dosage to adult male Wistar rats for ten days. This failed to produce any significant histochemical alterations in acid

phosphatase, B-glucuronidase, glucosaminidase or esterase activities in lysosomes of kidney or liver; nor were lysosomes in these locations altered in size or number. If, as suggested by deDuve (1963), lysosomes have a natural turnover in liver of 3% per day, then cortisone therapy failed to decrease natural loss, to a point where a quantitative increase in lysosomes could be detected histochemically.

(B) Phenacetin Treatment

Studer and Scharer (1965) administered large overdoses of phenacetin to beagle dogs over periods of 21 to 30 months. At the completion of this experiment they observed iron-positive and iron-negative pigments in the liver, and to a lesser extent in the kidney of experimental animals. Iron stroage was related to inclusion-body anemia with hemolysis. The iron-negative pigment displayed histochemical properties of lipofuscin. In another study, rats received phenacetin on a long-term basis; Torhorst et al. (1967) observed by electron microscopy that the deposits of pigment within renal tubular epithelial cells were of the nature of residual bodies derived from lysosomes and cytolysosomes. Essner and Novikoff (1960) have shown that lipofuscin pigment has a high acid phosphatase content. In the present study, rats received 1.8% phenacetin orally over a period of nine weeks. Histochemical staining reactions applied to kidneys from these animals failed to disclose any increase in size, number, or acid hydrolase activity in proximal renal tubules. No pigment granules were observed in unstained preparations. It must be assumed that the duration of the experiment was insufficient to induce large numbers of residual bodies with acid hydrolase activity in renal tubular epithelium.

IV. Effects of Phosphorus-Induced Hepatocellular Necrosis on Histochemically Demonstrable Lysosomal Hydrolases

deDuve (1963) has postulated that marginal damage to lysosomal membranes by hypoxia or other injurious agents may result in a release into the cytoplasm of lysosomal hydrolases with resultant necrobiosis. Yellow phosphorus is a potent hepatotoxin capable of producing peripheral zonal necrosis in liver. Barker, Smuckler, and Benditt (1963) have shown that the administration of yellow phosphorus to rats results in a displacement of ribosomes from endoplasmic membranes in liver, with an ensuing depression of protein synthesis.

In the experiment reported herein, yellow phosphorus suspended in mineral oil was administered to rats by stomach tube, as a single dose. Histochemical staining reactions showed a marked reduction of glucuronidase activity in proximal renal tubules, intercalated cells of collecting tubules, and cells of collecting ducts. Similarly, glucosaminidase activity was considerably reduced in lysosomes of intercalated cells. There was also complete inhibition of acid phosphatase activity in Kupffer cells of liver. In areas of hydropic degeneration and necrosis in liver lobules, there was no evidence of obvious diffusion of lysosomal enzymes into cytoplasm. Trump, et al. (1962), have noted that in areas of necrosis, of liver, lysosomes tend to maintain their structural integrity even when endoplasmic reticulum and mitochondria show established degenerative changes. It seems likely now that deDuve's suggestion that lysosomes initiate necrobiosis is an oversimplification of actual events. Depression of glucuronidase and glucosaminidase activities in kidney could either result from direct enzyme inhibition by residual phosphorus in tissue, or because of decreased

synthesis of enzymic protein on ribosomes. Histochemical techniques do not permit clarification of these points.

V. Concluding Remarks Concerning Experimental Results

It is generally not appreciated from biochemical data that variations in acid hydrolase content of tissues may variously result from increases or decreases in size, number, or specific activity of lysosomes. Moreover, the organelles involved may be primary lysosomes, phagolysosomes, cytolysosomes, or residual bodies. An attempt has been made in the experiments described and discussed herein, to deliniate these differences by the use of histochemical techniques. While histochemical methods provide precise topographical localization by light microscopy, they do not permit accurate quantitation or a morphologic distinction between categories of lysosomes. Because of these shortcomings, interpretable results were only obtained in two of eight experiments in which modulations were induced in populations of lysosomes. Testosterone propionate induces a 16-fold increase in B-glucuronidase activity in the kidney of the male mouse (Fishman et al., 1955, 1958). Inhibition of acid phosphatase activity in rat kidney and liver by N-2-fluorenylacetamide involves at least a 50% reduction in enzyme activity (Shibko, 1967). Only changes of the foregoing magnitude appear to fall into the range of histochemical sensitivity. Several hitherto unreported alterations in activities of lysosomal enzymes were noted on the basis of the precise topographical localization provided by histochemical techniques. One of these was evidence for selective inhibition of acid phosphatase in duodenal absorptive cells as opposed to macrophages in the lamina propria of the duodenum

following administration of N-2-fluorenylacetamide. Another example was the striking inhibition of glucuronidase in proximal renal tubules of rat following the administration of phosphorus. Both of these observations, however require confirmation and biochemical quantitation.

It would appear that histochemical methods under the foregoing circumstances are best suited to topographical surveys of enzyme activity. Semi-quantitation is only possible when experimentally induced changes in enzyme activity are of considerable magnitude. Morphologic characterization requires subsequent electron microscopy, while biochemical quantitation requires the use of both total homogenates and cell fractions.

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APPENDIX I. Biochemical Distribution of Acid Hydrolase Activities in Mammalian Tissues.

Survey of Published Results.

APPENDIX I. BIOCHEMICAL DISTRIBUTION OF ACID HYDROLASE ACTIVITIES IN MAMMALIAN TISSUES.

SURVEY OF PUBLISHED RESULTS.

TABLE (a) ENZYMES IN RAT AND SHEEP LIVER LYSOSOME FRACTIONS

(From Shibko, Caldwell, Savant, and Tappel, 1963)

ENZYME	RAT	SHEEP
Acid phosphatase	455 (64)	35 (12)
B-Galactosidase	0	46 (90)
Acid ribonuclease	264 (55)	398 (24)
Aryl sulphatase	15 (77)	410 (82)
B-Glucuronidase	610 (69)	390 (66)

Total enzyme activity is given in u moles/mg.N./mm. x 10^3.

Figures in parentheses represent % enzymes in bound form.

The lysosome fraction was a light mitochondrial fraction prepared by differential centrifugation.

TABLE (b) PARTICULATE DISTRIBUTION OF SOME HYDROLASES IN HOMOGENATES OF RAT LIVER.

(From Weissmann, Rowin, Marshall, and Friederici, 1967).

	Activity %		
Fraction	AP-ase	a-Acetyl Glucosaminidase	B-Acetyl Glucosaminidase
Nuclear	48±13	50±8	50±11
Cytoplasmic Extract (Heavy Mitochondrial)	(100)	(100)	(100)
Light Mitochondrial (lysosomal fraction)	44±7	44±5	57±5
Microsomal	28±6	18±2	23±5
Soluble	6±3	8±3	2±1

Rat liver homogenized in 0.25 M sucrose, and particulate fractions were collected by centrifugation (deDuve, et al., 1955). Numerical values designating activity are expressed relative to the cytoplasmic extract (supernatant fluid after removal of nuclear fraction) taken as 100%, plus or minus average deviation (in four experiments).

TABLE (c)

INTRACELLULAR DISTRIBUTION OF ENZYMES

(from Sellinger, et al., 1960)

Absolute values are given in mg./g. for nitrogen; in units /g. (fresh wt.) of rat liver for enzymes. Statistics refer to means ± S.D. These are given, rather than S.E.M., since they are a measure of individual variability, the most interesting information in the present case. E, cytoplasmic extract; N, nuclear fraction; M, heavy mitochondrial fraction; L, light mitochondrial fraction; P, microsomal fraction; S, final supernatant.

Enzyme	No. of Expt.	Absolute Values	Percentage values						
			E + N	N	M	L	P	S	Recovery
Nitrogen	5	31.5±5.6	100	20.8±5.0	17.4± 7.2	7.4± 4.0	19.8±2.9	37.1±9.2	102.5±5.9
Cytochrome oxidase	6	27.9±7.8	100	13.5±5.7	55.2±12.4	20.5±13.4	3.4±0.9	-	92.6±8.4
Acid phosphatase	6	4.88±0.8	100	9.9±4.1	20.8± 3.7	37.1± 5.1	24.4±3.0	11.9±3.7	104.1±5.7
B-N-Acetylglucosaminidase	4	6.90±1.1	100	12.4±4.6	33.4±16.4	39.4± 6.9	12.4±1.5	5.8±0.9	103.4±5.4
B-Galactosidase	2	0.34±0.08	100	7.6±5.6	29.4± 0.2	30.8± 1.4	10.9±1.1	10.8±3.0	89.5±2.1
Glucose 6-phosphatase	6	14.5±2.6	100	15.2±6.5	3.9± 1.5	6.0± 1.6	68.2±7.6	3.7±3.1	97.0±6.1

TABLE (d) SPECIFIC ACTIVITIES OF SOME ENZYMES OF RAT KIDNEY HOMOGENATE AND SUBCELLULAR FRACTIONS

(From Shibko and Tappel, 1967)

Results are specific activities of the enzyme. Values in parentheses indicate the ratios of the specific activity of each enzyme in the subcellular fraction to that in the homogenate. The number of experiments in the groups for which the S.D. is given was eight. All other results are the mean values of three experiments.

Enzyme	Homogenate	Lysosomal Fraction	Mitochondrial Fraction	Microsomal Fraction	Soluble Fraction
Protein (% of total homogenate)	100	0.36±0.12	11	6	28
AP-ase (B-glycerophosphate)	52.8±8.9	847 ±197(16)	212 (4.1)	74 (1.4)	15.8 (0.3)
AP-ase (p-nitrophenyl phosphate)	52	507(9.8)	146 (2.8)	82 (1.6)	40 (0.8)
Alkaline phosphatase	98	25(0.25)	31 (0.31)	395 (4.0)	5.6 (0.06)
Glucose 6-phosphatase	97	31(0.32)	23 (0.24)	284 (2.9)	4 (0.04)
Arylsulphatase	1.46±0.4	51.8±15.6(35)	14 (9.5)	0.9 (0.6)	0.9 (0.6)
Cathepsin (haemoglobin)	11.3±1.91	65±6.1 (5.8)	22 (1.9)	7.2 (0.6)	3.8 (0.34)
Cathepsin A	-	4.25	-	-	-
Cathepsin B	-	0.56	-	-	-
Cathepsin C	-	0	-	-	-
B-Glucuronidase	16.4±1.2	966±119(60)	176 (11)	6.6 (0.4)	102 (6.2)
B-Galactosidase	3.8±0.9	67.7±7.4(18)	14 (3.7)	1.8 (0.5)	2.9 (0.75)
a-Glucosidase	124.5±12.5	108±23.8(0.9)	43 (0.35)	205 (1.7)	8 (0.06)
Lysozyme	0.018±0.002	0.131±0.066(7.2)	0.03(1.7)	0.022(1.2)	0.007(0.4)
Acid ribonuclease	7.9±0.6	139.6±32.1	23 (2.9)	6.8 (0.86)	5.2 (0.65)
Glutamate dehydrogenase	17	11(0.62)	180 (10.5)	31 (0.18)	63 (3.7)
Succinoxidase	-	12	138	7.6	0
Catalase	375	23(0.06)	47 (0 12)	450 (1.2)	1460 (4)

TABLE (e) QUANTITATIVE DISTRIBUTION OF ACID HYDROLASES (from Woessner, 1965).

Tissue	Species	Substrate	Activity (u moles/hr./g.wet tissue)	References
		Acid Phosphatase		
Liver (L)	Rat	B-glycerophosphate	360	deDuve, et al., 1955
Liver	Rat	P-nitrophenyl phosphate	320	Barka, et al., 1961
Liver (L)	Mouse	B-glycerophosphate	103	Golberg, 1962
Liver (L)	Mouse	Phenyl phosphate	260	Golberg, et al., 1962
Liver (L)	Mouse	P-nitrophenyl phosphate	322	Golberg, et al., 1962
Spleen	Rat	P-nitrophenyl phosphate	408	Barka, et al., 1961
Spleen	Rat	B-glycerophosphate	88	Roth, et al., 1962
		B-glucuronidase		
Liver	Mouse		8.3	Conchie, et al., 1959
Liver	Rat		49.0	Conchie, et al., 1959
Spleen	Rat		48.0	Pellegrino & Villani, 1956
Spleen (L)	Rat		12.7	Conchie, et al., 1961
		B-galactosidase		
Liver	Mouse		29	Maio & Rickenberg, 1960
Liver	Mouse		10	Conchie, et al., 1959
Liver	Rat		34	Conchie, et al., 1959
Spleen	Mouse		57	Maio & Rickenberg, 1960
Spleen (male)	Mouse		55	Conchie, et al., 1959
Spleen (female)	Mouse		19	Conchie, et al., 1959
Spleen (male)	Rat		71	Conchie, et al., 1959
Spleen (female)	Rat		47	Conchie, et al., 1959
		Aryl sulfatase		
Liver (L)	Rat	Nitrocatechol Sulfate	207	Roy, 1958
Liver (L)	Mouse	Nitrocatechol Sulfate	55	Roy, 1958
		a-mannosidase		
Liver	Rat		13	Conchie, et al., 1959
Spleen	Rat		5.3	Conchie, et al., 1959
		Acetyl glucosaminidase		
Liver	Rat		370	Conchie, et al., 1959

APPENDIX II. Histochemistry of Lysosomal Acid Hydrolases.

Survey of Published Results.

APPENDIX II. HISTOCHEMISTRY OF LYSOSOMAL ACID HYDROLASES. SURVEY OF PUBLISHED RESULTS.

SPECIES	TISSUES	Acid phosphatase	B-glucuronidase	Glucosaminidase	Esterase	Aryl sulfatase	REFERENCES
Mammals							
Man	Jej.ep.	+					Riecken and Pearse, 1965.
	Endomet.	+					Cohen et al., 1964.
	Prostate	+	+		+		Maggi et al., 1966.
	Liver	+					Essner and Novikoff, 1961.
	Brain	+					Adams, 1965.
	Spleen	+			+		Stutte, 1967.
Dog	Cereb. cortex	+			+		Ogawa et al., 1961.
	Gall bladder	+					Salthouse et al., 1965.
Cat	Spinal cord	+					Koenig, 1962a.
		+					Barron and Tuncbay, 1964.
	Brain	+					Barron et al., 1966.
Rabbit	Panc.	+					Lazarus et al., 1965, 1966.
	Brain	+					Coimbra, 1964.
	Kidney	+					Ruyter, 1964.
	Liver	+					Ruyter, 1964.
	Spleen	+					Ruyter, 1964.
Hamster	Liver		+				Manning et al., 1964.
	Kidney		+				Manning et al., 1964.

SPECIES	TISSUES	Acid phosphatase	B-glucuronidase	Glucosaminidase	Esterase	Aryl sulfatase	REFERENCES
Guinea pig	Brain	+				+	Goldfischer, 1965.
	Kidney	+				+	Goldfischer, 1965.
	Liver	+				+	Goldfischer, 1965.
Mouse	Liver	+					de Man et al., 1960.
	Liver	+					Thorbecke et al., 1961.
	Liver	+					Trump and Ericsson, 1964.
	Liver					-	Woohsmann et al., 1964.
	Liver	+					Ericsson and Trump, 1965.
	Liver		+				Suzuki, 1965
	Liver	+					Maggi and Chng, 1966.
	Liver	+	+		+		Maggi et al., 1966.
	Kidney	+			+		Ostrowski et al., 1964.
	Kidney	+					Ruyter, 1964.
	Kidney					+	Woohsmann et al., 1964.
	Kidney		+				Suzuki, 1965.
	Spleen		+				Suzuki, 1965.
	Macrophages	+					Allison, 1965.
		+			+		Allison and Malluci, 1965.
	Duod.	+					Hugonand Borgers, 1965.
	Duod.	+					Hugon and Borgers, 1966.
	Jejun.	+					Barka, 1964.
	Brain					+	Woohsmann et al., 1964.
	Prostate	+	+		+		Maggi et al., 1966.
Rat	Liver	+					Holt, 1959.

SPECIES	TISSUES	Acid phosphatase	B-glucuronidase	Glucosaminidase	Esterase	Aryl sulfatase	REFERENCES
Rat	Liver	+					Essner and Novikoff, 1960.
	Liver	+					Barka et al., 1961.
	Liver	+					Essner and Novikoff, 1961.
	Liver				+		Shnitka and Seligman, 1961.
	Liver	+					Bitensky, 1962.
	Liver	+					Bitensky and Gahan, 1962.
	Liver	+					Novikoff and Essner, 1962.
	Liver	+			+		Holt, 1963.
	Liver	+			+		Sabatini et al., 1963.
	Liver	+					Allison and Malluci, 1964a.
	Liver		+				Hayashi et al., 1964.
	Liver		+				Hayashi, 1964.
	Liver	+					Straus, 1964.
	Liver	+					Ruyter, 1964.
	Liver					+	Woohsmann et al., 1964.
	Liver		+				Fishman and Goldman, 1965.
	Liver	+				+	Goldfischer, 1965.
	Liver			+			Hayashi, 1965.
	Liver		+				Suzuki, 1965.
	Liver	+					Meijer, 1966.
	Kidney	+					Holt, 1959.
	Kidney				+		Shnitka and Seligman, 1961.
	Kidney				+		Holt, 1963.
	Kidney	+					Morrison et al., 1963.
	Kidney	+			+		Sabatini et al., 1963.
	Kidney	+	+			+	Thomson and O'Connor, 1963.
	Kidney	+					Ericsson and Trump, 1964.
	Kidney		+				Hayashi et al., 1964.

SPECIES	TISSUES	Acid phosphatase	B-glucuronidase	Glucosaminidase	Esterase	Aryl sulfatase	REFERENCES
Rat	Kidney		+				Hayashi, 1964.
	Kidney	+					Ruyter, 1964.
	Kidney	+					Straus, 1964a and b.
	Kidney	+					Sandbank and Becker, 1964.
	Kidney					+	Woohsmann et al., 1964.
	Kidney	+					Ericsson and Trump, 1965.
	Kidney	+					Ericsson, 1965.
	Kidney		+				Fishman and Goldman, 1965.
	Kidney	+				+	Goldfischer, 1965.
	Kidney			+			Hayashi, 1965.
	Kidney		+				Suzuki, 1965.
	Kidney					+	Hopsu-Havu et al., 1967.
	Spleen	+					Barka et al., 1961.
	Spleen	+			+		Pettersen, 1964.
	Spleen	+					Ruyter, 1964.
	Spleen					+	Woohsmann et al., 1964.
	Spleen		+				Hayashi, 1964.
	Spleen	+				+	Goldfischer, 1965.
	Spleen		+				Suzuki, 1965.
	Brain	+					Barka et al., 1961.
	Brain	+					Koenig, 1962a.
	Brain	+					Koenig et al., 1964.
	Brain					+	Woohsmann et al., 1964.
	Brain	+					Sandbank and Becker, 1964.
	Brain	+					Anderson, 1965.
	Brain	+				+	Goldfischer, 1965.
	Brain	+					Torack, 1965.
	Panc.		+				Hayashi, 1964.

SPECIES	TISSUES	Acid phosphatase	B-glucuronidase	Glucosaminidase	Esterase	Aryl sulfatase	REFERENCES
Rat	Panc.					+	Woohsmann et al., 1964.
	Panc.	+				+	Goldfischer, 1965.
	Panc.	+					Sobel and Avrin, 1965.
	Intest.	+					Barka et al., 1961.
	Intest.		+				Hayashi, 1964.
	Intest.	+				+	Goldfischer, 1965.
	Duod.	+					Behnke, 1963.
	Jejun.	+					Ogawa et al., 1962.
	Capill. endoth.	+					Novikoff, 1961.

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